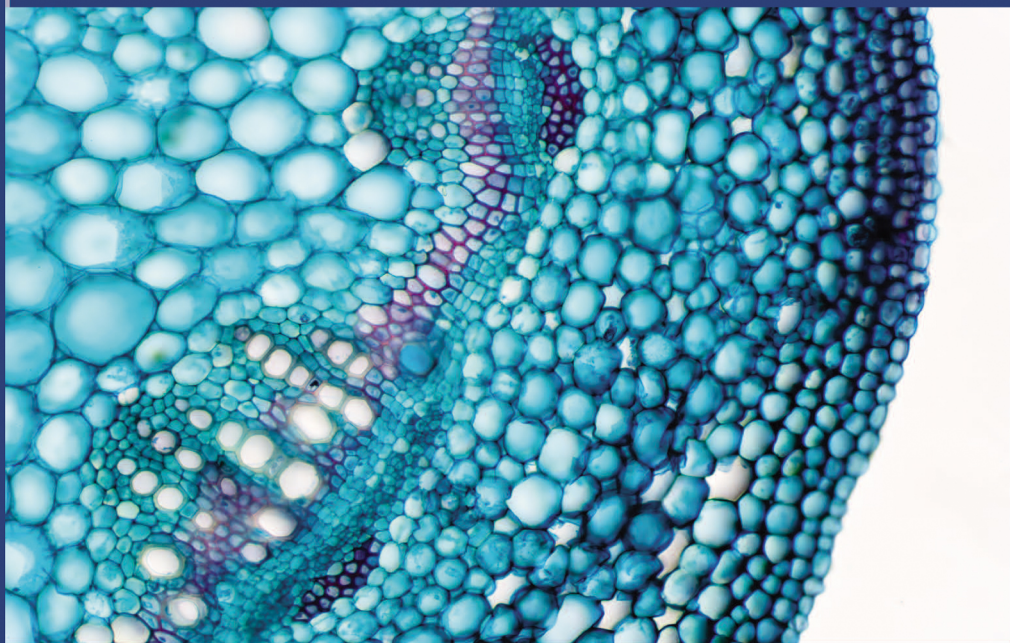


# Biology and Biotechnology of Environmental Stress Tolerance in Plants

*Volume 3*

Sustainable Approaches in Enhancing  
Environmental Stress Tolerance



Editor  
**Aryadeep Roychoudhury**



**CRC Press**  
Taylor & Francis Group

APPLE ACADEMIC PRESS

# **BIOLOGY AND BIOTECHNOLOGY OF ENVIRONMENTAL STRESS TOLERANCE IN PLANTS**

**Volume 3**

Sustainable Approaches for Enhancing  
Environmental Stress Tolerance

**Biology and Biotechnology of Environmental Stress Tolerance in Plants,  
Set of 3 Volumes**

ISBN: 978-1-77491-281-2 (hbk)

ISBN: 978-1-77491-282-9 (pbk)

**Biology and Biotechnology of Environmental Stress Tolerance in Plants,  
Volume 1: Secondary Metabolites in Environmental Stress Tolerance**

ISBN: 978-1-77491-283-6 (hbk)

ISBN: 978-1-77491-284-3 (pbk)

ISBN: 978-1-00334-617-3 (ebk)

**Biology and Biotechnology of Environmental Stress Tolerance in Plants,  
Volume 2: Trace Elements in Environmental Stress Tolerance**

ISBN: 978-1-77491-285-0 (hbk)

ISBN: 978-1-77491-286-7 (pbk)

ISBN: 978-1-00334-620-3 (ebk)

**Biology and Biotechnology of Environmental Stress Tolerance in Plants,  
Volume 3: Sustainable Approaches in Enhancing Environmental Stress  
Tolerance**

ISBN: 978-1-77491-287-4 (hbk)

ISBN: 978-1-77491-288-1 (pbk)

ISBN: 978-1-00334-640-1 (ebk)

# **BIOLOGY AND BIOTECHNOLOGY OF ENVIRONMENTAL STRESS TOLERANCE IN PLANTS**

**Volume 3**

**Sustainable Approaches for Enhancing  
Environmental Stress Tolerance**

*Edited by*

**Aryadeep Roychoudhury, PhD**

**AAP** | APPLE  
ACADEMIC  
PRESS



First edition published 2024

**Apple Academic Press Inc.**  
1265 Goldenrod Circle, NE,  
Palm Bay, FL 32905 USA  
760 Laurentian Drive, Unit 19,  
Burlington, ON L7N 0A4, CANADA

**CRC Press**  
6000 Broken Sound Parkway NW,  
Suite 300, Boca Raton, FL 33487-2742 USA  
4 Park Square, Milton Park,  
Abingdon, Oxon, OX14 4RN UK

© 2024 by Apple Academic Press, Inc.

*Apple Academic Press exclusively co-publishes with CRC Press, an imprint of Taylor & Francis Group, LLC*

Reasonable efforts have been made to publish reliable data and information, but the authors, editors, and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors, editors, and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged, please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, access [www.copyright.com](http://www.copyright.com) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. For works that are not available on CCC please contact [mpkbookspermissions@tandf.co.uk](mailto:mpkbookspermissions@tandf.co.uk)

Trademark notice: Product or corporate names may be trademarks or registered trademarks and are used only for identification and explanation without intent to infringe.

---

#### **Library and Archives Canada Cataloguing in Publication**

Title: Biology and biotechnology of environmental stress tolerance in plants / edited by Aryadeep Roychoudhury, PhD.

Names: Roychoudhury, Aryadeep, editor.

Description: First edition. | Includes bibliographical references and indexes. | Content: Volume 3: Sustainable Approaches in Enhancing Environmental Stress Tolerance.

Identifiers: Canadiana (print) 20230155588 | Canadiana (ebook) 20230155626 | ISBN 9781774912812 (set ; hardcover) | ISBN 9781774912829 (set ; softcover) | ISBN 9781774912874 (v. 3 ; hardcover) | ISBN 9781774912881 (v. 3 ; softcover) | ISBN 9781003346401 (v. 3 ; ebook)

Subjects: LCSH: Plant metabolites—Biotechnology. | LCSH: Plants—Effect of stress on. | LCSH: Plants—Adaptation.

Classification: LCC QK881 .B56 2023 | DDC 572/.42—dc23

#### **Library of Congress Cataloging-in-Publication Data**

.....

CIP data on file with US Library of Congress

.....

---

ISBN: 978-1-77491-287-4 (hbk)

ISBN: 978-1-77491-288-1 (pbk)

ISBN: 978-1-00334-640-1 (ebk)

## About the Editor

---



### **Aryadeep Roychoudhury, PhD**

*Assistant Professor, Department of  
Biotechnology, St. Xavier's College  
(Autonomous), Kolkata, India*

Aryadeep Roychoudhury, PhD, is Assistant Professor at the Department of Biotechnology, St. Xavier's College (Autonomous), Kolkata, West Bengal, India. He has over 22 years of research experience in the field of abiotic stress responses in plants with perspectives of physiology, molecular biology, and cell signaling under diverse stress conditions. Dr. Roychoudhury is currently handling several government-funded projects on abiotic stress responses in rice and supervising five PhD students as Principal Investigator. To date, he has published over 200 articles in peer-reviewed journals and chapters in books of international and national repute. He has edited many books from reputed publishers and has also guest edited several special journal issues. He is a regular reviewer of articles in high-impact international journals, a life member of several scientific associations and societies, and the recipient of the Young Scientist Award 2019, conferred by the International Foundation for Environment and Ecology, at the University of Allahabad, Prayagraj, Uttar Pradesh, India. His name is included in the Stanford University's List of World's Top 2% Scientists.

Dr. Roychoudhury received his BSc (Hons.) in Botany from Presidency College, Kolkata, and his MSc in Biophysics and Molecular Biology from the University of Calcutta, West Bengal, India. He did his PhD at the Bose Institute, Kolkata, under Jadavpur University, Kolkata, India.



# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

# Contents

---

<i>Contributors</i> .....	ix
<i>Abbreviations</i> .....	xiii
<i>Preface</i> .....	xxi

## **PART I: Microorganism Application and Stress Tolerance ..... 1**

- 1. Soil Microorganisms and Nematodes for Bioremediation and Amelioration of Polluted Soils.....3**  
Md. Basit Raza, Jyotirmaya Sahoo, Biswaranjan Behera, Ajin S. Anil, Rahul Kumar Tiwari, and Milan Kumar Lal
- 2. Rhizospheric Microbial Inoculation in Developing Stress Tolerance .....41**  
Suchismita Prusty, Monalisha Dasmohapatra, Sagar Maitra, and Ranjan Kumar Sahoo
- 3. Role of Endophytes, Plant Growth Promoting Rhizobacteria, and Arbuscular Mycorrhizal Fungi in Stress Tolerance..... 57**  
Anuron Banerjee, Nirmalya Chakraborty, Krishnendu Acharya, and Nilanjan Chakraborty

## **PART II: Priming and Genetic Engineering .....97**

- 4. Role of Hydropriming and Magneto-Priming in Developing Stress Tolerance.....99**  
Lekshmy Sathee, Meenakshi Thakur, Ravpreet Kaur, Sunita Kataria, Anjali Anand, Dalveer Singh, and Shailesh Tripathi
- 5. Magneto-Priming: A Novel Technique Towards Improved Seed Germinability and Stress Responses: From Basics to Contemporary Advancements ..... 133**  
Kuntal Bera, Subir Ghosh, Sanjoy Sadhukhan, and Puspendu Dutta
- 6. Plant Breeding Approaches in Developing Stress Tolerance.....167**  
Sami Ul-Allah, Asad Azeem, Ahmad Sher, Muhammad Ijaz, Abdul Sattar, Muhammad Naeem, and Imran Ullah

<b>7. Towards Engineering Smart Transcription Factors for Enhanced Abiotic Stress Tolerance in Plants .....</b>	<b>187</b>
Syed Sarfraz Hussain, Bushra Rasheed, Abdul Qayyum Rao, Ahmad Ali Shahid, and Bujun Shi	
<b>8. Genetics and Microarray in Environmental Stress Response .....</b>	<b>239</b>
Robab Salami, Masoumeh Kordi, Nasser Delangiz, Behnam Asgari Lajayer, and Tess Astatkie	
<b>9. Small RNAs – The Big Players in Developing Salt-Resistant Plants.....</b>	<b>249</b>
Anup Kumar Sarkar, and Sanjoy Sadhukhan	
<b>PART III: Epigenetics and Omics Approaches .....</b>	<b>303</b>
<b>10. Genome-Wide Association Studies and Next-Generation Sequencing in Plant Response to Environmental Stress .....</b>	<b>305</b>
Pooja Saraswat, Hunny Waswani, and Rajiv Ranjan	
<b>11. CRISPR/Cas and Its Potentiality as an Effective Tool in Understanding Environmental Stress Response .....</b>	<b>329</b>
Deepu Pandita	
<b>12. Epigenetics – The Molecular Tool in Understanding Abiotic Stress Response in Plants .....</b>	<b>349</b>
Kakan Ball and Sanjoy Sadhukhan	
<b>13. Advances in Proteomics Research in Environmental Stress Response in Plants.....</b>	<b>401</b>
Kasinathan Rakkammal, P. S. Jeevan Ram, and Manikandan Ramesh	
<b>14. Advances in Metabolomics Research in Environmental Stress Response in Plants .....</b>	<b>431</b>
Ankur Singh and Aryadeep Roychoudhury	
<b><i>Index.....</i></b>	<b><i>449</i></b>

# Contributors

---

## **Krishnendu Acharya**

Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany,  
University of Calcutta, Kolkata – 700019, West Bengal, India

## **Anjali Anand**

Division of Plant Physiology, ICAR–Indian Agricultural Research Institute, New Delhi – 110012, India

## **Ajin S. Anil**

Division of Soil Science and Agricultural Chemistry, ICAR–Indian Agricultural Research Institute,  
New Delhi, India

## **Tess Astatkie**

Faculty of Agriculture, Dalhousie University, Truro, NS B2N 5E3, Canada, E-mail: [astatkie@dal.ca](mailto:astatkie@dal.ca)

## **Asad Azeem**

College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Pakistan

## **Kakan Ball**

Plant Molecular Biology Laboratory, Department of Botany, Raiganj University,  
Raiganj – 733134, Uttar Dinajpur, West Bengal, India, E-mail: [ballkakan@gmail.com](mailto:ballkakan@gmail.com),  
ORCID: <https://orcid.org/0000-0001-9961-2805>

## **Anuron Banerjee**

Department of Botany, Scottish Church College, Kolkata – 700006, West Bengal, India

## **Biswaranjan Behera**

ICAR–Indian Institute of Water Management, Bhubaneswar, Odisha, India

## **Kuntal Bera**

Department of Seed Science and Technology, Uttar Banga Krishi Viswavidyalaya, Pundibari – 736165,  
Cooch Behar, West Bengal, India; Plant Molecular Biology Laboratory, Department of Botany,  
Raiganj University, Raiganj – 733134, Uttar Dinajpur, West Bengal, India,  
E-mail: [kuntalbera.dgc@gmail.com](mailto:kuntalbera.dgc@gmail.com), ORCID: <https://orcid.org/0000-0002-6879-6418>

## **Nilanjan Chakraborty**

Department of Botany, Scottish Church College, Kolkata – 700006, West Bengal, India,  
E-mail: [nilanjanchak85@gmail.com](mailto:nilanjanchak85@gmail.com)

## **Nirmalya Chakraborty**

Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany,  
University of Calcutta, Kolkata – 700019, West Bengal, India

## **Monalisha Dasmohapatra**

Department of Biotechnology, Centurion University of Technology and Management,  
Paralakhemundi – 761211, Odisha, India

## **Nasser Delangiz**

Department of Plant Biotechnology and Breeding, Faculty of Agriculture, University of Tabriz, Tabriz,  
Iran

**Puspendu Dutta**

Department of Seed Science and Technology, Uttar Banga Krishi Viswavidyalaya,  
Pundibari – 736165, Cooch Behar, West Bengal, India, E-mail: pdutta.pph@gmail.com,  
ORCID: <https://orcid.org/0000-0001-6659-8402>

**Subir Ghosh**

Plant Molecular Biology Laboratory, Department of Botany, Raiganj University, Raiganj – 733134,  
Uttar Dinajpur, West Bengal, India, E-mail: subir.ghosh302@gmail.com,  
ORCID: <https://orcid.org/0000-0003-3684-7265>

**Syed Sarfraz Hussain**

Kausar Abdulla Malik School of Life Sciences (KAM-SLS), Forman Christian College  
(A Chartered University), Ferozepur Road, Lahore – 54600, Pakistan

**Muhammad Ijaz**

College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Pakistan

**Sunita Kataria**

School of Biochemistry, DAVV, Indore, Madhya Pradesh, India

**Ravpreet Kaur**

Division of Genetics, ICAR–Indian Agricultural Research Institute, New Delhi, India

**Masoumeh Kordi**

Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology,  
Shahid Beheshti University, Tehran, Iran

**Behnam Asgari Lajayer**

Health and Environment Research Center, Tabriz University of Medical Science, Tabriz, Iran,  
E-mail: [asgarihemayat@gmail.com](mailto:asgarihemayat@gmail.com)

**Milan Kumar Lal**

Scientist (Plant Physiology), Division of Crop Physiology, Biochemistry, and PHT,  
ICAR–Central Potato Research Institute, Shimla, Himachal Pradesh, India,  
E-mails: [milan2925@gmail.com](mailto:milan2925@gmail.com); [milan.lal@icar.gov.in](mailto:milan.lal@icar.gov.in)

**Sagar Maitra**

Department of Agronomy and Agroforestry, Centurion University of Technology and Management,  
Paralakhemundi – 761211, Odisha, India

**Muhammad Naem**

Department of Plant Breeding and Genetics, UCA&ES, The Islamia University of Bahawalpur, Pakistan

**Deepu Pandita**

Government Department of School Education, Jammu, Jammu and Kashmir, India,  
E-mail: [deepupandita@gmail.com](mailto:deepupandita@gmail.com), ORCID: <https://orcid.org/0000-0002-8361-1872>

**Suchismita Prusty**

Department of Biotechnology, Centurion University of Technology and Management,  
Paralakhemundi – 761211, Odisha, India

**Kasinathan Rakkammal**

Department of Biotechnology, Science Campus, Alagappa University, Karaikudi – 630003,  
Tamil Nadu, India

**P. S. Jeevan Ram**

Department of Biotechnology, Science Campus, Alagappa University, Karaikudi – 630003,  
Tamil Nadu, India



**Manikandan Ramesh**

Department of Biotechnology, Science Campus, Alagappa University, Karaikudi – 630003, Tamil Nadu, India, E-mail: mrbiotech.alu@gmail.com

**Rajiv Ranjan**

Dayalbagh Educational Institute, Department of Botany, DayalBagh, Agra – 282005, Uttar Pradesh, India, E-mail: rajivranjanbt@gmail.com

**Abdul Qayyum Rao**

National Center of Excellence in Molecular Biology (NCEMB), West Canal Bank Road, Thokar Niaz Baig, University of The Punjab, Lahore, Pakistan

**Bushra Rasheed**

National Center of Excellence in Molecular Biology (NCEMB), West Canal Bank Road, Thokar Niaz Baig, University of The Punjab, Lahore, Pakistan

**Md. Basit Raza**

Division of Soil Science and Agricultural Chemistry, ICAR–Indian Agricultural Research Institute, New Delhi, India

**Aryadeep Roychoudhury**

Post-Graduate Department of Biotechnology, St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Kolkata – 700016, West Bengal, India, E-mail: aryadeep.rc@gmail.com

**Sanjoy Sadhukhan**

Plant Molecular Biology Laboratory, Department of Botany, Raiganj University, Raiganj – 733134, Uttar Dinajpur, West Bengal, India, E-mail: sanjoysadhukhan@gmail.com, ORCID: <https://orcid.org/0000-0002-2619-8700>

**Jyotirmaya Sahoo**

Division of Soil Science and Agricultural Chemistry, ICAR–Indian Agricultural Research Institute, New Delhi, India

**Ranjan Kumar Sahoo**

Department of Biotechnology, Centurion University of Technology and Management, Paralakhemundi – 761211, Odisha, India, E-mail: ranjan.sahoo@cutm.ac.in

**Robab Salami**

Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

**Pooja Saraswat**

Dayalbagh Educational Institute, Department of Botany, DayalBagh, Agra – 282005, Uttar Pradesh, India

**Anup Kumar Sarkar**

Department of Botany, Dukhulal Nibaran Chandra College, Aurangabad – 742201, Murshidabad, West Bengal, India; Plant Molecular Biology Laboratory, Department of Botany, Raiganj University, Raiganj – 733134, Uttar Dinajpur, West Bengal, India, ORCID: <https://orcid.org/0000-0002-6418-4895>

**Lekshmy Sathee**

Division of Plant Physiology, ICAR–Indian Agricultural Research Institute, New Delhi – 110012, India, E-mail: lekshmyrnair@gmail.com

**Abdul Sattar**

College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Pakistan

**Ahmad Ali Shahid**

National Center of Excellence in Molecular Biology (NCEMB), West Canal Bank Road, Thokar Niaz Baig, University of The Punjab, Lahore, Pakistan

**Ahmad Sher**

College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Pakistan

**Bujun Shi**

School of Agriculture, Food, and Wine, University of Adelaide, SA – 5064, Australia

**Ankur Singh**

Post-Graduate Department of Biotechnology, St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Kolkata – 700016, West Bengal, India

**Dalveer Singh**

Division of Genetics, ICAR–Indian Agricultural Research Institute, New Delhi, India

**Meenakshi Thakur**

College of Horticulture and Forestry, Dr. Y.S. Parmar University of Horticulture and Forestry, Neri, Himachal Pradesh, India

**Rahul Kumar Tiwari**

ICAR–Central Potato Research Institute, Shimla, Himachal Pradesh, India

**Shailesh Tripathi**

Division of Genetics, ICAR–Indian Agricultural Research Institute, New Delhi, India

**Sami Ul-Allah**

College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Pakistan,  
E-mail: samipbg@bzu.edu.pk

**Imran Ullah**

Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

**Hunny Waswani**

Dayalbagh Educational Institute, Department of Botany, DayalBagh, Agra – 282005, Uttar Pradesh, India

# Abbreviations

---

μT	microtesla
7mG	7-methylguanosine cap
ABA	abscisic acid
ABPP	activity-based protein profiling
ac	acetyl
AC	alternating current
ACC	1-aminocyclopropane-1-carboxylate
AFLP	amplified fragment length polymorphism
AGO	argonaute
Al	aluminium
AM	Arbuscular mycorrhizae
AMF	arbuscular mycorrhizal fungi
AO	amine oxidase
AOX	alternative oxidase
APEX	ascorbate peroxidase
APX	ascorbate peroxidase
AQP	aquaporins
ArcA2	autosomal recessive cerebellar ataxia 2
As	arsenic
AsA	ascorbic acid
ATFs	artificial transcription factors
B	boron
BADH1	betaine aldehyde dehydrogenase 1
bHLH	basic helix-loop-helix
BioID	dependent biotin identification
BN-PAGE	blue native-polyacrylamide gel electrophoresis
bZIP	basic leucine zipper
C	carbon
Ca	calcium
CA	carbonic anhydrase
cAMP	responsive element-binding protein
cAPX	cytosolic ascorbate peroxidase
casiRNA	cis-acting siRNAs
CAT	catalase

CBF	C-repeat-binding factors
Cd	cadmium
CD	chromodomain
CDK	cyclin-dependent protein kinase
CDPK	calcium-dependent protein kinase
CE-MS	capillary electrophoresis mass spectrometry
ChIP	chromatin immunoprecipitation
ChIP-Seq	ChIP-sequencing
Chl-a	chlorophyll-a
Cl <sup>-</sup>	chloride
Cl	chlorine
CLCuKoV	cotton leaf curl Kokhran virus
CMT3	chromomethylase3
CMV	cucumber mosaic virus
Co	Cobalt
CO <sub>2</sub>	carbon dioxide
COR	cold-responsive
CP	coat protein
CpTi	cowpea trypsin inhibitor
Cr	chromium
CRISPR-Cas9	clustered regularly interspaced short palindromic repeat-associated protein 9
crRNA	CRISPR RNA
CRY2	cryptochrome circadian regulator 2
CS	Chinese spring
CSD	copper/zinc superoxide dismutase
CSPs	cold shock-domain family proteins
Cu	copper
CWP	cell wall protein
DArT	diversity arrays technology
DBD	DNA binding domains
DC	direct current
dCas9	dead CRISPR-associated protein 9
DCL	dicer-like
DEG	differentially expressed genes
DESI	desorption electrospray ionization mass spectrometry
DHA	dehydroascorbic acid
DHAR	dehydroascorbate reductase
DHN	dehydrins
DIMS	direct infusion mass spectrometry

DML2	demeter-like 2
DON	deoxynivalenol
DPI	diphenylene iodonium
DREB2A	dehydration-responsive element binding protein 2A
DRM	domains rearranged methylase
ds RNA	double-stranded RNA
DSB	double-strand break
EC	electrical conductivity
ECM	extracellular matrix
EMF	electromagnetic field
EPS	extracellular polymeric molecules
ER	endoplasmic reticulum
ERFVII <sub>s</sub>	ethylene response factors VII
EST	expressed sequence tags
FAO	Food and Agriculture Organization
Fe	iron
FFE	free flow electrophoresis
FI-ICR-MS	Fourier transform ion cyclotron resonance mass spectrometry
FnCas9	<i>Francisella novicida</i> Cas9
GA	gibberellic acid
GLM	general linear model
GM	genetically modified
GMF	geomagnetic field
GMStool	GWAS-based marker selection tool
GOI	genes of Interest
GOLS3	galactinol synthase 3
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
GS-MS	gas chromatography-mass spectrometry
GSS	genome survey sequences
GST	glutathione-S-transferase
GWAS	genome-wide association study
H	hydrogen
H2A	histone 2A
H2B	histone 2B
H2O2	hydrogen peroxide
H3	histone 3
H4	histone 4

HAT	histone acetyltransferase
HCN	hydrogen cyanide
hc-siRNA	heterochromatic siRNA
HD2D	histone deacetylase 2D
HDAC	histone deacetylases
HDGS	homology-related gene silence
HDM	histone demethylases
HDR	homology-directed repair
HEN1	HUA enhancer1
HIGS	host-induced gene silencing
HKT1	high-affinity K <sup>+</sup> transporters 1
HM	heavy metal
HMA	heavy metal-transporter P-type ATPases
HMT	histone methyltransferase
HPTLC	high-performance thin-layer chromatography
HR	homologous recombination
HS	heat stress
HsfA1s	heat shock transcription factor A1s
HSP	heat shock protein
HSR	heat stress response
Hz	hertz
IAA	indole acetic acid
IDS1	indeterminate spikelet1
ISR	induced systematic resistance
JA	jasmonic acids
K	potassium
KDa	kilodalton
kHz	kilohertz
LCM	laser capture microdissection
LC-MS	liquid chromatography-mass spectrometry
LD	linkage disequilibrium
LEA	late embryogenesis abundant
LOX	lipoxygenases
LSD1	lysine-specific demethylase 1
MALDI	matrix-assisted laser desorption ionization
MAPK	mitogen-activated protein kinase
MBF1c	multiprotein bridging factor 1C
MDA	malondialdehyde
MDH	malate dehydrogenase

MDHAR	monodehydroascorbate reductase
me	methyl
MeDIP	methylated DNA immunoprecipitation
mEq/L	milliequivalents per liter
MET1	methyltransferase 1
MF	magnetic field
mg L <sup>-1</sup>	milligram per liter
Mg	magnesium
MIR	mycorrhiza-induced resistance
miRNA	microRNAs
MLM	mixed linear model
MLO	mildew locus O
Mn	manganese
Mo	molybdenum
mT	miliTesla
MTA	marker-trait associations
MTW	magnetic-treated water
N	nitrogen
Na <sup>+</sup>	sodium
NAD	nicotine adenine dinucleotide
nat-siRNA	natural antisense siRNA
ncRNA	noncoding RNAs
NGS	next-generation sequencing
NHEJ	non-homologous end joining
Ni	nickel
NMR	nuclear magnetic resonance
NO	nitric oxide
NOS	nitrogen oxygen species
NOX	NADPH oxidase
NPR-1	non-expression of pathogenesis-related
NR	nitrate reductase
nT	nanotesla
O <sub>2</sub>	oxygen
O <sub>2</sub> <sup>-</sup>	superoxide
ODC	ornithine decarboxylase
OEE	oxygen-evolving enhancer protein
ORM	organelle-locatable reactive molecules
P	phosphorus
P5CDH	pyrroline-5-carboxylate dehydrogenase



PAH	polycyclic aromatic hydrocarbon
PAL	phenylalanine ammonia-lyase
PAM	protospacer adjacent motif
PAST	pathway association tool
Pb	lead
PCB	polychlorinated biphenyls
PCD	programmed cell death
PCDD/PCDF	polychlorinated dibenzofurans
PCR	polymerase chain reaction
PDEs	phosphodiesterases
PEG	polyethylene glycol
PGPB	plant growth-promoting bacteria
PGPM	plant growth-promoting microorganisms
PGPR	plant growth promoting rhizobacteria
ph	phosphate
PHD	plant homeodomain
piRNA	piwi-interacting RNAs
PMF	pulsed magnetic field
POD/POX	peroxidase
POP	persistent organic pollutants
PPO	polyphenol oxidase
PR	pathogenesis-related
PRC2	polycomb repressive complex2
pri-miRNA	primary miRNA
PRK	phosphoribulokinase
PS	photosystem
PSPDB	plant stress protein database
PTGS	post-transcriptional gene silencing
PTI	PAMP-triggered immunity
PTM	post-translational modification
PWWP	pro-Trp-Trp-Pro
qPCR	quantitative PCR
QTL	quantitative trait loci
RanBP	ran-binding protein
RAPD	random amplified polymorphic DNA
RdDM	RNA-directed DNA Methylation
RDP3/HDA1	reduced potassium dependency 3/histone deacetylase 1
RDRs	RNA dependent RNAs
RFLP	restriction fragment length polymorphism

RIL	recombinant inbred line
RISC	RNA-induced silencing complexes
RITS	RNA-induced transcriptional silencing
RNA Pol-II	RNA polymerase II
RNAi	RNA interference
RNAP	RNA polymerase
RNAPIV	RNA polymerase IV
RNS	reactive nitrogen species
ROS	reactive oxygen species
RRBS	reduced-representation bisulfite sequencing
S	sulfur
SA	salicylic acid
SAM	S-adenosyl L methionine
SAR	systemic acquired resistance
SBPase	sedoheptulose-1,7-bisphosphatase
sgRNA	single guide RNA
SHMT	serine hydroxymethyltransferase
SIR2	silent information regulator 2
siRNA	short interfering RNAs
SMF	static magnetic field
SNF	sucrose non-fermenting
SNP	single nucleotide polymorphisms
SNP	sodium nitroprusside
SOD	superoxide dismutase
sRNA	small RNA
sRNA-seq	sRNA Sequencing
SSNs	sequence-specific nucleases
ssRNA	single-stranded RNA
SSRs	simple sequence repeats
STIFDB	stress-responsive transcription factor database
st-RNA	small temporal RNA
TALE	transcription activator-like effectors
tasiRNA	trans-acting siRNAs
TCA	tricarboxylic acid cycle
TCE	trichloroethylene
TDS	total dissolved solids
TE	transposable element
TF	transcription factor
TGN	trans-Golgi network

TGS	transcriptional gene silencing
THS	terminal heat stress
TK	transketolase
TMV	tobacco mosaic virus
TNT	trinitrotoluene
TPH	total petroleum hydrocarbon
tracrRNA	trans-activating crRNA
tru-gRNA	truncated gRNAs
TuMV	turnip mosaic virus
ub	ubiquitin
UPLC	ultra-high performance liquid chromatography
UV-B	ultraviolet B
VAM	vesicular arbuscular mycorrhiza
VDAC	voltage-dependent anion channel
WGBS	whole-genome bisulfite sequencing
XO	xanthine oxidase
ZF	zinc finger
Zn	zinc
β-CAS	β-cyanoalanine synthase

# Preface

---

Terrestrial plants, since their emergence, are subjected to harsh environmental conditions that include hostile factors such as temperature extremes, water deficit or excess, heavy metal toxicity, salinity, UV radiation, etc. Owing to their sessile nature, plants need to confront such stresses, which adversely affect their life cycle and their vital physiological processes like seed germination, seedling growth, flowering, and seed production. Therefore, it is not only necessary to understand the biochemical and molecular mechanisms underpinning the defense responses, but also to come up with effective strategies to mitigate stress-mediated toxicity and ensure better yield. A lot of progress has been witnessed in understanding of the forward and reverse genetic approaches and genome-wide analyses, especially in model plants like the classical *Arabidopsis thaliana* and its extremophyte relative *Thellungiella salsuginea*, both exhibiting multi-stress tolerance.

Traditional agronomic practices, including selection of tolerant cultivars, fertilizer management, mutation breeding through radiation exposure, and pre-treatment of seeds and seedlings before sowing, include some of the effective adaptive strategies to fight against such stresses. The first rice varieties, KT 20–74 and SH 30–21, released in China as early as 1957, were developed through induced mutation. A semi-dwarf and non-lodging mutant variety of wheat called Sharbati Sonora was generated through the treatment of a red-grained Mexican variety, Sonora 60, by gamma radiation. Many abiotic stress-tolerant crops, including varieties of rice, wheat, corn, potato, soybean, cotton, and sugarcane, with superior yield, have been successfully produced by mutation breeding and are being used for human consumption in many countries. Priming of seeds and seedlings, viz., pre-exposure to water and protective chemical agents, induces “stress memory” existing in both the present generation and its offspring, and therefore appears as a promising strategy to cope with climate change and improve plant performance. Various compounds like phytohormones, growth regulators, and signaling molecules are induced during stress, each of which when used as a priming agent, enables the plants to adapt against adverse situations. Stress memory is highly epigenetic-based and involves diverse mechanisms, including DNA methylation, histone modification, nucleosome positioning, chromatin remodeling, and noncoding RNA-mediated regulation. Identification of

appropriate strains of root-colonizing, non-pathogenic, plant growth-promoting bacteria and plant inoculation with such microbial consortium has been found to provide cross-protection against multiple environmental stressors like drought, salinity, and metal toxicity and enhance the efficacy of phytoremediation of inorganic and organic pollutants.

Genetic and biotechnological methods have been developed over time immemorial to develop stress-tolerant plants. Advanced conventional breeding strategies are required to expedite the generation of stress-tolerant varieties. Such strategies heavily rely on certain pillars like large mapping populations in the form of recombinant inbred lines, double haploids, and near-isogenic lines, marker-assisted selection, marker-trait association, mapping software, and identification of genes by linkage mapping and quantitative trait loci. The introduction of a single gene or gene pyramiding with multiple genes via transgenic technology, is always a widely opted approach by scientists to develop stress-resilient plants. The transgenic approach allows the identification of the target genes, transcription factors, and microRNAs, providing an integrated knowledge of the physiological and molecular mechanism of stress tolerance. Recent developments in omics approaches and systems biology, coupled with next-generation sequencing (NGS), epigenomics, and CRISPR/Cas technology, also have a high implication in the future in developing climate-resilient plants. Omics platforms like genomics, transcriptomics, proteomics, and metabolomics have provided an opportunity to gather a holistic knowledge of stress-mediated regulation for plant defense. Systems biology coordinates the information from various high throughput omics tools, thereby enabling us to comprehend the mechanism of plant response. Small RNA (siRNA and miRNA) technology acts as a nodal player of plant development at both post-transcriptional and transcriptional levels by regulating gene expression and combating various abiotic stresses. The noncoding RNAs and their putative target genes show differential expression patterns during stress, implying epigenetic regulation of noncoding RNA expression. Apart from DNA methylation, post-transcriptional RNA modifications are also regarded as plant epigenetic regulators. Targeting the regulatory or structural genes or cis-regulatory sequences and creating novel QTL through robust genome editing tools like CRISPR/Cas9 has allowed a rapid and precise modification of any organism at the nucleotide level. Another emerging field is nanotechnology, where nanoparticle application has highly improved plant performance by improving the free radical scavenging and antioxidant potential during stress and has revolutionized the agricultural sector.

The third volume, entitled “Sustainable Approaches for Enhancing Environmental Stress Tolerance,” of this 3-volume publication book, therefore, focuses on the agronomic and biochemical approaches as well as biotechnological and high throughput technologies, including the prospects of genetic engineering, epigenetics, and the latest CRISPR/Cas technology in generating stress-tolerant plants. I am sure that this volume will definitely provide a clear road map with regard to the implementation of different techniques for improving abiotic stress tolerance in plants for better sustenance.



# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>



**PART I**

**Microorganism Application and  
Stress Tolerance**



# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

## CHAPTER 1

---

# Soil Microorganisms and Nematodes for Bioremediation and Amelioration of Polluted Soils

MD. BASIT RAZA,<sup>1</sup> JYOTIRMAYA SAHOO,<sup>1</sup> BISWARANJAN BEHERA,<sup>2</sup>  
AJIN S. ANIL,<sup>1</sup> RAHUL KUMAR TIWARI,<sup>3</sup> and MILAN KUMAR LAL<sup>3\*</sup>

<sup>1</sup>*Division of Soil Science and Agricultural Chemistry,  
ICAR–Indian Agricultural Research Institute, New Delhi, India*

<sup>2</sup>*ICAR–Indian Institute of Water Management, Bhubaneswar, Odisha, India*

<sup>3</sup>*ICAR–Central Potato Research Institute, Shimla, Himachal Pradesh, India*

*\*Corresponding author.*

*E-mails: milan2925@gmail.com; milan.lal@icar.gov.in*

---

## ABSTRACT

Widespread industrialization and anthropogenic activities have increased the accumulation of toxic pollutants that includes most importantly heavy metals that pose a serious threat to human and aquatic lives. Industries being the major source of environmental pollution are indiscriminately releasing toxic and hazardous wastes (both organic and inorganic) resulting in worsening of the existing situation. In order to mitigate the toxic effect of heavy metals, there are a large array of physical and chemical methods were adapted for remediation of these metals from the soil and water bodies. These techniques, however, are costly and energy-intensive, because secondary air or groundwater contamination, and can affect soil microbial diversity, making bioremediation a more desirable option. The method of bioremediation comprises the use of microorganisms which include the

class of autochthonous or zymogenous that secretes enzymes that lead to the breakdown of the heavy metal compounds. Moreover, these microbes are also helpful to detoxify environmental pollutants. It is efficient in reducing toxic pollutants concentration below that causing human health risk, high in cost-effective ratio and minimal/no-secondary pollution generation issues. However, the efficiency of this method is severely limited by the unavailability of the right strain of microbe and various environmental factors like oxygen concentration, temperature, nutrient, and co-substrates availability and presence of potentially toxic compounds. Our present chapter includes the problem associated with the toxicity pollutant which includes both organic and inorganic pollutants. Moreover, we also tried to include a sustainable, viable, and eco-friendly bioremediation technology which are microbes-assisted bioremediation. Further, it would highlight the current emerging issues and challenges, which require consideration for the future development of novel technologies for the bioremediation of contaminated sites.

## **1.1 INTRODUCTION**

The recent trends of industrialization and rapid urbanization worldwide has led to the incidence of xenobiotic contamination in soil and air. This contamination of heavy metals and organic pollutants might result in nutrient runoff, soil erosion, and ultimately lead to the pollution of soil and water bodies. Increased incidence of soil pollution in terms of contamination of soil by the enhanced release of the harmful substance (heavy metal and organic pollutants) into the soil affect the flora and fauna of the soil. However, unlike the air pollution that directly affect the lives of animal and human, soil pollution can indirectly and detrimentally affect the lives of all form (Mishra, Mohammad, & Roychoudhury, 2016). The primary sources of heavy metal include mining and smelting of metallic ores, fertilizers, municipal; wastes, sewage sludge, pesticides, burning of fossil fuels and electronic wastes (Devi et al., 2021; Mondal et al., 2019). Both intended and unintended activities can result in soil pollution, which broadly is of two types *viz.* point source and diffuse pollution. The main sources of point-source pollution are anthropogenic activities and are very common in urban areas. Diffuse pollution occurring in the soils is difficult to analyze, and examples include the use of pesticides in agriculture, soil erosion, uncontrolled disposal of waste, persistent organic fertilizers and fertilizers that also add heavy metals (Wu & Chen, 2013). Remediation involves removing toxic or biohazardous contaminants

that can have possible human health hazards or threats to the environment from an infected area. Remediation is a cleanup and disinfecting process. Conventional methods of remediation followed ‘cap and contain’ strategy, i.e., digging up and removing it to a landfill and then maintaining it on the same land. In this case, the products barely lose their hazardous nature as they stay in their original form but in isolation. However, in recent times with the development of advanced techniques these pollutants could be destroyed, if possible, be transformed into harmless substances. However, they have major drawbacks *viz.* lack effectiveness, high cost of application (expensive), technological complexity, lack of public acceptance, and more secondary toxic compounds being released during the incineration process.

Bioremediation (bio = living, remediate = to bring the sites and affairs into the original states) makes an effective better sustainable approach possible to achieve amelioration of polluted soils (Arora, 2018). The term bioremediation is defined as any process which uses the biological system to reduce the toxic effect of the pollutants. The biological system might include microorganisms, green plants, fungi, or enzymes derived from the biological system that reduces the contaminant concentration in soil (Schaechter, 2009). As microorganisms are easy to manage and plants are easy to grow, bioremediation is superior to the use of conventional methods for remediation purposes. The other terms which are generally used for the process of bioremediation are bio-reclamation, biotreatment, and bio-restoration. It can be carried out *in situ* or in advanced reactors (*ex-situ*). Bioremediation by microorganisms and nematodes requires a good atmosphere for the polluted sites to be cleaned up. The process of microbial activity in the polluting sites might require the addition of extra components so that the microbes can thrive well. These extra components include the addition of nutrients, favorable temperature, moisture, and terminal electron acceptor ( $O_2/NO_2$ ) that help to enhance the growth of a specific organism (Lal et al., 2022; Altaf et al., 2022).

Soil microorganisms and nematodes can degrade pollutants consisting predominantly of heavy metals, hydrocarbons, oils, polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) as they have the capacity to mitigate heavy metals and also decompose well after its effect on microbes (Jariyal et al., 2020; Meena et al., 2022; Tiwari et al., 2021). However, bioremediation is not readily used for the treatment of all pollutants; certain heavy metals, such as Pb and Cd are not readily absorbed or captured by microorganisms and nematodes (Kumar et al., 2011). It is a natural alternative mechanism, rather than a panacea to strategies such as

incineration, catalytic destruction, adsorbent use, physical removal, and eventual pollutant destruction. There is, however, a distance between laboratory research developments and commercial field applications. Therefore, the present chapter focuses on the use of various soil microorganisms and nematodes as a possible strategy that can contribute very effectively and in an eco-friendly way for bioremediation and amelioration of polluted soils. Further, it also delineates the challenges and limitations hindering the widespread application of these clean green approaches involving microbes.

## **1.2 SOURCES AND TYPES OF POLLUTANTS IN THE ENVIRONMENT AND THEIR FATE**

The present scenario of high anthropogenic activity affected the environment which is the primary issue of the society. The pollutant that is naturally occurring and foreign matter significantly affected the environment and created adverse conditions for soil and water bodies. They all attract substantial attention due to the environmental impacts they trigger. There are many pollutants in soil, therefore the main pollutants in the soil can be broadly categorized as of three types, i.e., organic, inorganic pollutants, and biological.

### **1.2.1 ORGANIC POLLUTANTS**

The accrual of organic pollutants in the environment has an immense impact on the solidarity of both land and marine habitats, creating more detrimental effects on human health (UNEP, 2015). The wastes from the kitchen and garden are generally categorized as biodegradable waste and mostly are of organic types. However, the waste from the factories and agricultural runoff are categorized as persistent organic pollutants (POPs). Stockholm convention classified 12 POPs into:

1. **Industrial Chemicals:** Polychlorinated biphenyls (PCBs) and hexachlorobenzene.
2. **Pesticides:** Endrin, Heptachlor, Hexachlorobenzene, Mirex, Aldrin, Chlordane, DDT, Dieldrin, Toxaphene.
3. **By-Products:** Polychlorinated dibenzofurans (PCDD/PCDF), hexachlorobenzene, polychlorinated dibenzo-p-dioxins and PCBs.

These aforementioned chemicals were distinguished by the low solubility rate and high lipid solubility, which makes it possible for them to bioaccumulate in animal fatty tissues and join the food chain (Mondal et al., 2019).

### **1.2.2 INORGANIC POLLUTANTS**

Inorganic pollutants are typically chemical substances of salts and minerals derived from manufacturing, mineral origin, such as metals, agricultural, and domestic waste (Masindi & Muedi, 2018). These inorganic pollutants are naturally occurring but reported to be transformed by human production to increase their amount contained in the environment. Heavy metal toxicity is one of the toxic components among the inorganic pollutants, and it is a major threat to the food chain, which leads to major environmental and health issues in the present modern society.

### **1.2.3 BIOLOGICAL POLLUTANTS**

Biological pollutants are primarily the product of human activities and have a significant effect on both aquatic ecosystems and the terrestrial. In particular, it is the introduction of non-indigenous and xenobiotic species (Masindi & Muedi, 2018). A wide diversity of pollutants is found in nature, which can be classified as in Table 1.1.

## **1.3 APPROACHES FOR REMEDIATION OF ENVIRONMENTAL POLLUTANTS**

Apparently, bioremediation methods can be classified as *in situ* (at the site) or *ex situ* (away from the site), taking into account the site of operation. Some of the environmental factors to be considered while selecting any bioremediation technique are listed in Table 1.2.

### **1.3.1 BIOREMEDIATION AT IN SITU**

The bioremediation at *in situ* level signifies when there is detoxification of the pollutant precisely where the pollution has occurred. The remediation process does not need excavation or extraction of soil or water.



**TABLE 1.1** Classification of Pollutants Present in the Environment

Pollutants	Classification with Examples			References
Organic	Halogenated	Pesticides	BHC, DDT, and derivatives (dicofol, DFDT, etc.), cyclo-dienes (endosulfan, heptachlor, chlordane, aldrin, dieldrin, endrin, etc.), others (mirex, toxaphene)	UNEP (2015)
		Solvents	DCM, chloroform, etc.	–
		Other	PCBs, dioxins, hexachlorobenzene, PAHs, CFCs, HFCs, HCFCs, etc.	–
	Non-halogenated	Non-halogenated hydrocarbons	Petroleum hydrocarbons, dimethyl phthalate, explosives (nitroaromatic explosive), and solvents viz. BTX (benzene, toluene, xylene), etc.	Mondal et al. (2019)
Inorganic	Metal/metalloids	Mostly heavy metals and toxic agrochemicals	As, Cd, Pb, Hg, Se, pesticides (Hg, Cu, As, etc.) and fertilizers (micronutrient based), and salts	Masindi & Muedi (2018)
	Non-metals	Toxic non-metals	Cyanides, ammonium, sulfur, salts, etc.	Fuller (1977)
	Radioactive	Am, Be, <sup>32</sup> P, <sup>14</sup> C, <sup>15</sup> N, <sup>59</sup> Fe, <sup>3</sup> H, <sup>238</sup> U, <sup>232</sup> Th, and <sup>210</sup> Po, etc.		Gulan et al. (2017)
Biological	This category of pollutants includes bacteria, viruses, molds, mildew, animal dander and cat saliva, house dust, mites, cockroaches, and pollen.			UNEP (2015)

**TABLE 1.2** Optimum Conditions for Bioremediation of Environmental Pollutants

Environmental Factor	Conditions Required for Microbial Activity	References
Available soil moisture	25–85% water holding capacity	Harekrushna & Kumar (2012); Haghollahi, Fazaelipoor, & Schaffie (2016)
Oxygen	Aerobic, minimum air-filled pore space of 10%	
Soil type	Low clay or silt content	
pH	5.5–8.5	
Temperature	15–45°C	

The detoxification or remediation of the site requires oxygen and nutrient by circulating the polluted soil to facilitate the degradation of the organic pollutant by the naturally occurring bacteria. The process of bioremediation was reported to be the cheapest and most effective method for detoxification of heavy metals in soil (Kumar et al., 2011). *In situ* bioremediation is of mainly two types: (i) intrinsic *in situ* bioremediation; and (ii) engineered *in situ* bioremediation.

### 1.3.1.1 INTRINSIC/NATURAL ATTENUATION IN SITU BIOREMEDIATION

It uses the biodegradation of toxic pollutants to harmless substances by the innate capacity of the microorganisms already present in the environment (Vidali, 2001). In this process, there is no interference of humans, and it is considered the cheapest method of bioremediation which is widely known. Scientists turn to engineered bioremediation when the intrinsic bioremediation is not feasible under certain circumstances.

### 1.3.1.2 ENGINEERED/ENHANCED IN SITU BIOREMEDIATION

The aforementioned process requires the introduction of the microbes into the site of the contamination. Moreover, in this method, there may be the addition of engineered microbes for the bioremediation process. This enhances and accelerates the process of degradation of heavy metals by improving the physiochemical condition and growth of microbes. Mainly, electron acceptors, nutrients, and oxygen are added along with nitrogen and phosphorus to enhance the development of the microbes (Rodríguez et al., 2009). Some important *in situ* bioremediation processes are discussed hereunder.

#### **1.3.1.2.1 Bioventing**

The process of bioventing is an approach to aerating the water and soil that accelerate the process of degradation of organic substances (Hyman & Dupont, 2001). The aforementioned method is used mainly for the purpose of the treatment of the unsaturated soils and enables aeration in the soil. This method supplies oxygen to the microbes which are present in the water and soil, thereby accelerating the activity of microbes. This process also enhances the growth of the indigenous microbes that help in the decomposition of organic matter. This methodology helps to enhance the degradation of the anaerobically degradable polluted soil which are rich in organic hydrocarbon, pesticides, and other chemicals (Dupont, 1993; Khan, Husain, & Hejazi, 2004). However, this process is not successful if the water table is situated within a few feet under the soil (Van Deuren et al., 2002).

#### **1.3.1.2.2 Biosparging**

The aforementioned method is generally used to degrade the products which are mainly composed of petroleum and also dissolved in water. The majority of the petroleum product is absorbed by the water table in soil, so this methodology is very much helpful (Norris, 1993, 1995). In this method, the atmospheric air is injected through the soil which is both saturated and unsaturated soil. To have the application of this method efficiently, the soil should be homogenous in the polluted region. The biosparging method has also been used to reduce energy consumption (Dary et al., 2010).

#### **1.3.1.2.3 Bioaugmentation**

The process of bioaugmentation is achieved by the addition of laboratory-grown microbes which has the potential for degradation of the heavy metal pollutant. In the aforementioned process, the microbial strain with higher Polycyclic Aromatic Hydrocarbon (PAH)-degrading capabilities were inoculated in the polluted soil environment (Dhanya & Kalia, 2020). This approach introduces either wild type or genetically engineered microbes which help to enhance the degradation of heavy metal and reclaim the soil (Kuiper et al., 2004). There are various advances in the bioaugmentation method which includes gene bioaugmentation, cell encapsulation, phyto-augmentation, and rhizosphere bioaugmentation (Gentry, Rensing, & Pepper, 2004).

#### **1.3.1.2.4 Phytoremediation**

Phytoremediation is an efficient process, and it is cheap and environmental friendly in the removal of heavy metal and toxic material from the soil. This method requires the involvement of plant and their part which is supported by the allied rhizospheric microorganism to extract and remove the pollutant (Hooda, 2007). Generally, a heavy metal such as Cd, Se, and As is used to be removed from the soil (contaminated soil) which are generally present in the form of metal and metalloid (Hooda, 2007). This sustainable and inexpensive process is evolving rapidly as a viable alternative to traditional remediation approaches and would be the most acceptable for developing countries like India. Despite this feasibility, phytoremediation has yet to become a potential commercial technology available in India (Ghosh & Singh, 2005).

#### **1.3.1.2.5 Subsurface Bioremediation**

Bioremediation did at the subsurface region of soil where both organic and inorganic pollutants. Ethylbenzene and xylenes (BTEX), benzene, toluene, bioremediation is done by this method (Shim & Yang, 1999).

#### **1.3.1.2.6 Free Enzyme Bioremediation**

This is a newly developed technology allowing the rapid detoxification of residues of pesticides in surface waters, such as irrigation water, and probably from other wettable products, such as soil and product surfaces (Scott et al., 2011).

### **1.3.2 EX SITU BIOREMEDIATION**

#### **1.3.2.1 SOLID-PHASE SYSTEM**

Composting is a method that involves the mixing of organic compounds such as agricultural waste with polluted soil. The presence of the organic material in the soil leads to the creation of a higher microbial population in the soil which might lead to a higher composting temperature (Shilev et al., 2007). However, this method is a more controlled and extended version of the land farming.

### *1.3.2.2 LAND FARMING OPERATION*

This is a basic technique of excavating and spreading polluted soil and preparation of bed by regular tiling until the pollutant in the soil gets degraded. The aforementioned process is limited, and the handling of the soil was done at 10–35 cm, on the superficial surface. It is the most efficient in eliminating PAH and PCP.

### *1.3.2.3 BIOWASTE SYSTEM*

It is a combination of composting and land cultivation. In this process, the engineered cell is designed as composted aerated piles. It is also used for the treatment of petroleum hydrocarbons and the extraction of pollutants from the surface. This process is the modification of land farming which aims to monitor the physical losses of the pollutant that leads to leaching and volatilization (Williams, 2006). It provides indigenous aerobic and anaerobic microorganisms with a congenial habitat for their proliferation.

### *1.3.2.4 SLURRY PHASE SYSTEM*

Contaminated materials, microorganisms, and water are formulated into slurry within the bioreactor or fermentor. The bioreactors are designed to promote microbial degradation.

### *1.3.2.5 USE OF GENETIC ENGINEERING*

The use of DNA technology among the various forms of biotechnology has the potential of producing new combinations of genes that have not existed in nature before. The use of restriction enzymes has enabled the isolation of particular DNA fragments that can be transferred to another organism lacking the same. Genes that code for environmental pollutant metabolism, such as PCBs and other xenobiotic compounds, are often found on plasmids, but not always. A modern viewpoint on bio-waste treatment has opened up the possibility of genetic transfer to non-biodegrading microbes (Sayler & Ripp, 2000). The recombinant DNA has the potential to multiply and may grant the unique derivative capacity to detoxify environmental pollutants.

#### 1.3.2.6 BIOFILMS

It consists of an organized population of bacterial, algal, or other cell types contained in a polymer matrix that is self-produced. Waste can accumulate within biofilm to toxic levels. Used for treatment of recalcitrant compounds.

#### 1.3.2.7 BIOLEACHING

Involves extraction of desired metal from low-grade ores with the help of microorganisms. Microorganisms produce acids from reduced sulfur compounds, and the acids solubilize the desired metals.

### 1.4 MICROBIAL BIOREMEDIATION: A GREEN TECHNOLOGY FOR ENVIRONMENTAL REMEDIATION

The industrial revolution in recent decades enabled humans to explore and exploit natural resources which leads to the deterioration of the environment and human health. For dealing with environmental pollution, bioremediation is the most novel and ideal solution for pollution abatement. This approach uses biological systems for the treatment of contaminants.

#### 1.4.1 MICROORGANISMS INVOLVED IN BIOREMEDIATION

##### 1.4.1.1 BACTERIA

Bacteria are the most important microbes in the clean-up of contaminated soil and water because they break up dead material into inorganic nutrients very quickly. Different pesticides including chlorinated pesticides can easily be digested by bacteria. Bacteria can act on both aerobic and anaerobic functions by using the enzymatic pathway and catabolic reaction of microorganisms to break down soil contaminants or convert them into nontoxic substances.

##### 1.4.1.1.1 Aerobic Bacteria

The variety of anaerobic bacteria is recognized for the ability to degrade which includes *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. The aforementioned bacteria were reported to degrade and decay the pesticide and hydrocarbon which are generally alkanes and

polyaromatic compounds. The mechanism behind the detoxification is that many bacteria use the pollutant as the sole source of carbon and energy. Bacteria such as *Mycobacterium*, *Escherichia*, and *Bacillus* are commonly used for the bioremediation of heavy metals and PAHs. The mechanism of use of PAHs as the source of carbon as they break down the substances like anthracene, pyrene, naphthalene, phenanthrene, and benzopyrene in the presence of heavy metals such as Cd, Cu, Cr, and Pb that are reported to have occurred with PAHs. The enzyme used by the microbes for aerobic degradation is methane monooxygenase which acts against a wider range of compounds that includes chlorinated aliphatic trichloroethylene and 1,2-dichloroethane.

#### **1.4.1.1.2 Anaerobic Bacteria**

There use isn't as prevalent as aerobic bacteria. These are primarily used for the bioremediation of the polychlorinated biphenyls (PCBs) which are present in the river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform.

#### **1.4.1.2 FUNGI**

The group of fungus was reported to be used as the bioagent for bioremediation of soil. The fungi such as the white-rot fungus *Phanaerochaete chrysosporium* was reported to have the ability to degrade the extremely diverse range of pollutants and heavy metals. The growth of the fungus was accelerated by the addition of straw, sawdust, and corn cobs where it acts as the substrate. Moreover, the heavy metal pollutant might also be remediated by the fungal communities such as *Pleurotus*, *Acremonium*, and *Fusarium*. However, the microbial consortia were reported to be associated with the effective strategies for remediation of heavy metals and PAHs. There are some well-established techniques where the process of immobilization (Huang et al., 2016) and compost (Poulsen & Bester, 2010) can protect the microbes from heavy metals and PAHs, thereby enhancing the activity of microorganisms.

#### **1.4.1.3 ALGAE**

Several species of algae have shown the ability to biosorb organic and inorganic pollutants, specifically heavy metals and even radioactive substances.

Quite recently, the use of the extraction from algae in the remediation of wastewater has recently gained interest from the scientific community which is reported to have great potential as it fixes carbon dioxide (CO<sub>2</sub>). Moreover, the algae biomass also contributed to the feedstock for biofuel production (Huang et al., 2010). Moreover, the recent study by Yamamoto et al. (2008) suggested that the microalgae were used first time for the remediation of organically enriched sediments. Algae is also useful for the wider range of organic pollutants which includes PAHs, petroleum hydrocarbons, polychlorinated biphenyls (PCBs), explosives like trinitrotoluene (TNT) and pesticides. In addition to it, algae are also used for the biomonitoring and restoration of the aquatic system and lead to having favor in the phytoextraction and biodegradation of many organic pollutants available in the soil. Moreover, complex formation Ion exchange, and electrostatic interaction aid in the biosorption of heavy metal ions by algae (Zeraatkar et al., 2016).

#### **1.4.2 GENERAL MECHANISMS FOR MICROBIAL BIOREMEDIATION**

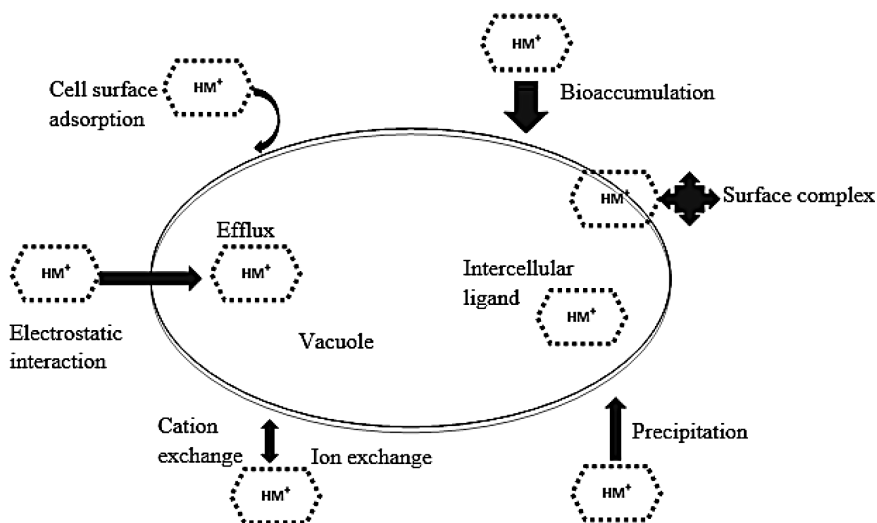
In the presence of xenobiotic substances, which have the potential to damage environmental health and functioning, microorganisms use a variety of ways to interact and survive. As far as the degradation of organic pollutants are concerned, these rather act as carbon source for microbes which are consumed either in the presence or absence of oxygen producing less or non-toxic substrate products. Quite naturally, rapid, and complete degradation of pollutants is brought under aerobic condition. The key reaction of aerobic biodegradation is oxidation, which is catalyzed by oxygenase and peroxidases. Various groups of organisms, such as denitrifiers, methanogens, and sulfidogens, are actively involved in the bioremediation process under anaerobic bacteria circumstances.

By combining fermenters and acetogens, as well as methanogens and sulfate reducers, carbon molecules are decomposed step by step, creating methane, CO<sub>2</sub>, ammonia, and hydrogen sulfide. In most cases, anaerobic degradation mechanisms have been deemed inferior to aerobic degradation. Anaerobic procedures, on the other hand, have proven to be efficient and significantly less expensive than aerobic treatment when substantial loads of easily degraded organic components are present. Microbes with a variety of methods, including metal-organic complexation, metal-ligand degradation, oxidation, methylation, enzymatically, metal efflux pumps, intracellular, and extracellular metal sequestration, and metal exclusion, reclaim heavy metals



as the most common inorganic pollutants (Jaganathan et al., 2018; Awadhesh et al., 2020; Chourasia et al., 2022; Mangal et al., 2022). Metals, especially at high concentrations, can harm the microbial population by interfering with metabolic processes such as cell division suppression, protein denaturation and cell membrane rupture. Tolerant microorganisms demonstrate the ability to live in the presence of high heavy metal(loid) concentrations by utilizing survival strategies such as biotransformation, extrusion, enzyme usage, generation of exopolysaccharide (EPS), and metallothionein synthesis (Chinnusamy, Zhu, & Sunkar, 2010; Lal et al., 2020; Devi et al., 2021; Awadhesh et al., 2021).

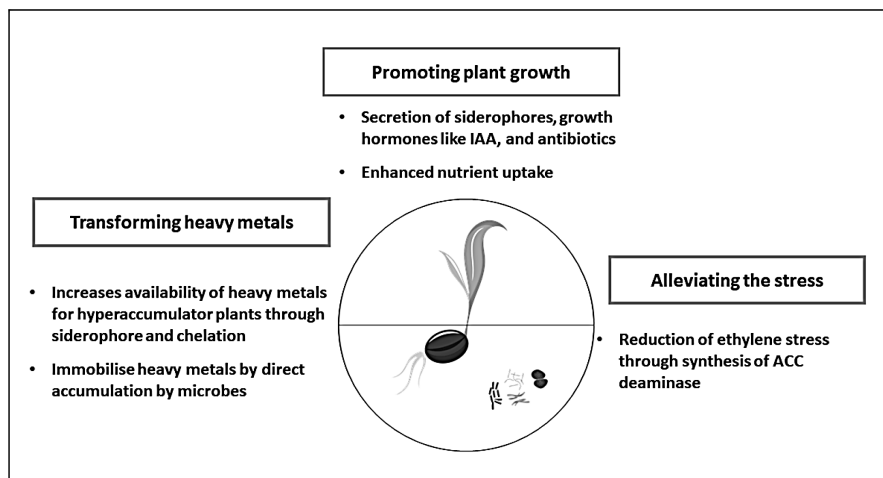
Microbe transformation of metal(loid)s could be broadly categorized into two types, redox conversions (oxidation and reduction) to less toxic state and conversions from inorganic to organic form and vice versa. Oxidation of metal(loid)s could help gain energy and on the contrary reduction can occur through dissimilatory reduction where microorganisms utilize metal(loid)s as terminal electron acceptor for anaerobic respiration. The reduction process mainly could be methylation and demethylation. Microbial methylation is quite important because of volatile products formed during the process leading complete phase change of inorganic contaminants from solid (soil) to gas (atmosphere) resulting in complete amelioration. A pictorial representation of a microbe cell imparting several mechanisms of tolerance to high heavy metal(loid) ( $\text{HM}^+$ ) concentration is presented in Figure 1.1.



**FIGURE 1.1** Mechanism of microbial detoxification of heavy metals.

### 1.4.3 MICROBIAL CONSORTIUM OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) FOR BIOREMEDIATION

Microorganisms and plants are used in bioremediation, which is a widely acknowledged and successful *in-situ* technique of treating heavy metal contaminated soils. A plant-based phytoremediation is a typical approach of contaminated soil bioremediation. Bioremediation that incorporates both microbes and plants provides a more efficient clean-up of heavy metal-polluted soils. The effectiveness of this strategy, however, is highly dependent on the type and efficiency of the organisms involved. Plant growth-promoting rhizobacteria (PGPR) are bacteria that colonize the rhizosphere of plants and stimulate plant development (Kloepper, 1978). Heavy metals may be removed from the environment through phytostabilization, phytoextraction, rhizofiltration, and phytovolatilization (Kumar et al., 2021). The PGPR has the potential to enhance the phytoremediation of heavy metals through promoting plant growths, transforming heavy metals, and/or alleviating heavy metal stress (Zhuang et al., 2007). A conceptual model for the role of PGPR in phytoremediation of heavy metals has been depicted in Figure 1.2.



**FIGURE 1.2** Mechanism of bioremediation of heavy metals through PGPR.

The interaction between arbuscular mycorrhizal fungi (AMF) and PGPR have a synergistic effect on plant due to channelization of rhizospheric P (Bhardwaj et al., 2014). Mishra et al. (2016) reported enhanced uptake of Fe by plants in Fe contaminated soil under combined action of AMF and PGPR

(Mishra et al., 2016). Nannipieri et al. (2007) identified higher interactions among rhizospheric microbes in metal contaminated soil (Nannipieri et al., 2007). Several reports are available on the potential of biochar (a porous carbonaceous material produced by pyrolysis of crop residues) in heavy metal remediation (Guimarães et al., 2021; He et al., 2019). The use of PGPR and biochar along with a hyperaccumulator plant significantly increased the Cd content and bioaccumulation factor of accumulator, about 412% and 403% higher than that of control, respectively (Wu et al., 2019). This system also increased the fresh and dry biomass of the metal accumulator plant by 227.27% and 178.33%, respectively. Khan & Bano (2016) identified catalase and oxidase enzymes secreted from the PGPR isolated from municipal wastewater that solubilize insoluble bound phosphate and exhibit antifungal and antibacterial activities. The use of Ag-nanoparticles with these PGPR enhanced the levels of abscisic acid (ABA) by 34%, indole acetic acid (IAA) by 55%, gibberellin by 82%, and proline synthesis by 70%, as well as alleviating oxidative stress and enhancing the bioremediation capacity of PGPR for Pb, Cd, and Ni. Table 1.3 summarizes the function of PGPR in the remediation of heavy metal-polluted soil. Biofilms have been used to remove heavy metals, according to studies (Yin et al., 2019). Biofilms are characterized as bacterial communities attached to a surface or to each other inside a matrix (Poulsen, 1999; Moussa & Algamal, 2017). Harekrushna & Kumar (2012) investigated bacterial biofilm zinc sorption and the role of extracellular polymeric molecules (EPS).

The microbial EPS can bind with heavy metals and protect the cells from the hostile environment. Forming biofilms by these strains is considered a natural strategy to maintain a favorable niche in stressful environments with increased metals concentrations. Costley & Wallis (1999) reported the efficiency of biofilm in removing the heavy metals in order of Cu>Zn>Cd with the removing efficiency of 73%, 42%, and 33% respectively. Workentine et al. (2008) reported that metal toxicity might be reduced by biofilm by altering their physiology for protection of sensitive chemical targets against the reactive metal species.

## **1.5 ENDOPHYTIC MICROBES ASSISTED BIOREMEDIATION**

Natural remediation techniques provide more environment friendly opportunity for cleanup of the polluted soils and water. Phytoremediation due to its cost-effectiveness has caught attention worldwide. It uses the natural

**TABLE 1.3** Role of PGPR in Bioremediation of Heavy Metals

Heavy Metal	Plant Species	Microorganisms	Growth Condition	Mechanism of Remediation	References
Fe <sup>3+</sup>	<i>Pennisetum glaucum</i> and <i>Sorghum bicolor</i>	Fungi ( <i>Glomus</i> , <i>Acaulospora</i> , and <i>Scutellospora</i> ) and PGPR ( <i>Streptomyces</i> , <i>Azotobacter</i> , <i>Pseudomonas</i> , and <i>Paenibacillus</i> ) association	Pot experiment	Increased Fe uptake by plants through siderophore formation	Mishra et al. (2016)
Cr <sup>5+</sup> and Cd <sup>2+</sup>	<i>Lepidium sativum</i>	<i>Azotobacter</i> sp.	Lab experiment	Stimulated plant growth and enhanced tolerance to heavy metals	Sobariu et al. (2017)
Cd	<i>Chrysopogon zizanioides</i>	<i>Serratia marcescens</i> SNB6 fixed with biochar	Pot experiment	Enhanced the Cd content and bioaccumulation factor of the plant	Wu et al. (2019)
Pb, Cd, and Ni	<i>Zea mays</i>	<i>Pseudomonas</i> sp., <i>Pseudomonas fluorescens</i> , and <i>Bacillus cereus</i> with Ag-nanoparticles	Pot experiment	Solubilization of insoluble bound phosphate and plant growth promotion	Khan & Bano (2016)
Zn and Pb	None	Biofilm of <i>Pseudomonas aeruginosa</i> (P8) and three strains of <i>P. fluorescens</i> (P4, P9, P10)	Culture media	Immobilization of heavy metals from the solution	Oladipo et al. (2018)
Cd	<i>Oryza sativa</i>	<i>Enterobacter aerogenes</i> K6	Pot experiment	Reduced Cd uptake and oxidative stress through IAA production, P solubilization, ACCD activity	Pramanik et al. (2018)
As	<i>Oryza sativa</i>	One strain of <i>Pseudomonas</i> sp. (S6) and two strains of <i>Bacillus</i> sp. (S7 and S10)	Green house and field condition	Promoted plant growth and reduced As uptake	AW et al. (2020)

Note: ACCD: 1-aminocyclopropane-1-carboxylate deaminase.

ability of plants to purify soil and water bodies of potentially harmful toxic elements (organic and inorganic). This method provides a huge advantage over conventionally used physical or chemical technologies in terms of efficiency, environmental sustainability, cost-effectiveness, and ecology enhancement. However, several limitations such as phytotoxicity, slow degradation, and limited uptake of contaminant, evapotranspiration of volatile contaminants and disposal of plant residue constrain the application of this technology (Khan & Doty, 2011). Majority of these limitations can be overcome by plant-associated microbes (bacteria, fungi, and actinomycetes) or endophytes which can improve the amelioration of some pollutants. Endophytes reside in the living plants sharing a mutualistic relationship without causing apparent negative symptoms of infection (Huo et al., 2012).

Heinrich Friedrich Link, a German botanist, was the first to characterize endophytes as a unique category of partially parasitic fungus that live in plants in 1809 (Kumar & Saxena, 2020). *Enterobacteriaceae*, *Pseudomonadaceae*, and *Burkholderiaceae* are some of the most common endophyte genera. Endophytic bacteria have a variety of advantages over rhizospheric bacteria in phytoremediation. Rhizospheric bacterial populations are difficult to regulate, and competition between rhizospheric bacterial strains frequently lowers the number of the desired strain. Quantitative gene expression of xenobiotic catabolic genes and genetic modification of the catabolic pathway may aid in improving the efficacy of plant-based bioremediation.

### **1.5.1 ENDOPHYTE AIDED PHYTOREMEDIATION OF INORGANIC POLLUTANTS**

Extracellular precipitation, intracellular accumulation and sequestration, biotransformation of hazardous metal ions to less or non-toxic forms, and adsorption/desorption of metal ions are all methods by which endophytic bacteria might lower metal phytotoxicity (Ma et al., 2016). The genes that code for metal or antibiotic resistance proteins can help to relieve or remove abiotic or biotic stress. Sun et al. (2010) discovered that endophytic bacteria could modulate the activity of plant antioxidant enzymes (such as POS, CAT, SOD, glutathione (GSH) peroxidase, and ascorbate peroxidase (APX)) as well as lipid peroxidation, which confronted plant defense mechanisms, and that this could help plants resist heavy metal-induced oxidative stress.

Furthermore, methylation can be used by certain endophytic bacteria as a metal resistance or detoxifying process. Some mercury-resistant endophytic

bacteria, for example, express the MerB gene, which encodes organo-mercuriallyase, which converts organomercurials to mercuric ion ( $\text{Hg}^{2+}$ ) (Brown et al., 2003). Luo et al. (2011) reported that the cadmium resistant endophytic bacterium *Serratia* sp. LRE07 was able to absorb approximately 65% of Cd and 35% of Zn in bacterial cells from a single metal solution, considerably lowering the phytotoxic effects of the metals by sharing the metal burden.

The metal biosorption by bacteria can be facilitated passively or actively. In passive uptake metal binds with functional groups on the cell surface which could be by precipitation, chelation, or ion exchange (Schiewer & Volesky, 2014). While in active biosorption or bioaccumulation, the metals can be metabolically taken up inside the bacterial cell. Endophytes are reported to alter the bioavailability and uptake of metals into plant by secreting a variety of metabolites, including siderophores, organic acids (e.g., citric, oxalic, and acetic acids), etc., thus avoiding phytotoxicity (Visioli et al., 2014; Tiwari et al., 2021; Lal et al., 2021). Barzanti et al. (2007) reported that 81% of bacterial isolates recovered from *Alyssum bertolonii* were shown to produce siderophores and to promote plant growth under Ni stress.

When copper-resistant endophytic bacteria were introduced into a host plant, they showed strong Cu translocation from the root to the shoot of *Brassica napus*, resulting in a reduction in Cu hyperaccumulation overall (Sun et al., 2010). Toxic metals and metalloids are immobilized by fungal endophytes, which create metal oxalate or trigger chelation onto melanin-like polymers. By over-expressing As translocation factor, vesicular-arbuscular mycorrhiza (VAM) can boost arsenic (As) absorption in the hyperaccumulating fern *Pteris vittata* L. (Trotta et al., 2006). *Mucor* sp. and endophytic yeasts (*Cryptococcus* sp. CBSB78 and *Rhodotorula* sp. CBSB79) were identified from *Brassica chinensis* L. growing in metal-rich soil, with the potential to increase Pb, Zn, copper (Cu), and cadmium (Cd) bioaccumulation (Deng et al., 2011; Wang et al., 2013). Table 1.4 lists several commonly identified endophytes that helped with heavy metal phytoremediation.

### **1.5.2 ENDOPHYTE AIDED PHYTOREMEDIATION OF ORGANIC POLLUTANTS**

Numerous research has shown the efficacy of endophyte-assisted phytoremediation in the removal of organic contaminants from soil. Even at extremely low concentrations, the presence of stubborn organic pollutants in the environment limits plant and microorganism development and metabolic

**TABLE 1.4** List of Different Endophytes Involved in Phytoremediation of Organic and Inorganic Pollutants from Soil

Endophyte Microorganism	Host Plant	Pollutant	Mechanism of Action	References
<b>(a) Inorganic Pollutants</b>				
<i>Bacillus</i> sp. L14	<i>Solanum nigrum</i>	Cd and Pb removal up to 80.48% and 75.78%, respectively.	Induced hormesis by heavy metals	Guo et al. (2010)
<i>Pseudomonas koreensis</i> AGB-1	<i>Miscanthus sinensis</i>	Inoculated plants showed relatively higher heavy metal(loid) content viz. As, Cu, Cd, Zn, and Pb over uninoculated plants	Solubilization of heavy metal(loid)s causing high uptake by hyperaccumulator; promotion of plant growth	Babu et al. (2015)
<i>Rahnella</i> sp. JN27	<i>Amaranthus hypochondriacus</i> and <i>A. mangostanus</i>	Hyperaccumulation of Cd by host plant even at very high Cd concentrations	Production of siderophores, IAA, ACC deaminase, and the Cd solubilization in soils	Yuan et al. (2014)
<i>Penicillium funiculosum</i> LHL06	<i>Glycine max</i> L.	Pb, Al, Cr, Ni, and Cu accumulation in shoots of soybean decreased by 23.1%, 42.6%, 5.2%, 68.2%, and 90.1%, respectively, compared to that of non-inoculated plants	Extracellular adsorption or intracellular absorption of heavy metals by LHL06	Bilal et al. (2019)
<i>Lindgomycetaceae</i> P87	<i>Aeschynomene fluminensis</i>	Soil Hg decreased by 57% in <i>A. fluminensis</i> plants inoculated with P87	Volatilization and bioaccumulation of Hg in plant tissues	Pietro-Souza et al. (2020)
<i>Mucor</i> sp. MHR-7	<i>Brassica campestris</i> L.	The strain removed 60–87% of heavy metals from broth culture when supplied with 300 $\mu\text{g mL}^{-1}$ of the metals.	Absorption of heavy metals in mycelia prevented its uptake by plant	Zahoor et al. (2017)
<b>(b) Organic Pollutants</b>				
<i>Pseudomonas putida</i> M1441 (pNAH7)	<i>Pisum sativum</i>	Inoculated plants showed higher (40%) naphthalene degradation rates compared to uninoculated plants	Phytoprotection, phytoremediation	Germaine et al. (2006)

**TABLE 1.4** (Continued)

Endophyte Microorganism	Host Plant	Pollutant	Mechanism of Action	References
<i>Burkholderia</i> sp. HU001, <i>Pseudomonas</i> sp. HU002	Willow	Degradation of toluene was improved leading to a decreased toxicity and evapotranspiration	Biodegradation, production of siderophores, organic acids, and IAA	Weyens et al. (2010)
<i>Enterobacter ludwigii</i> strains viz. ISI10-3 and BRI10-9	Italian ryegrass, birdsfoot trefoil and alfalfa	Both showed degradation rates of diesel fuel up to 68%	Hydrocarbon degradation and ACC deaminase activities	Yousaf et al. (2011)
<i>Phomopsis</i> <i>liquidambari</i>	Rice	Combination with rice decreased phenanthrene up to 25.68% in plant compared with uninoculated treatment	Higher accumulation and biodegradation	Fu et al. (2018)
<i>Neotyphodium</i> <i>coenophialum</i> , <i>N.</i> <i>uncinatum</i>	<i>Festuca arundinacea</i> ; <i>F. pratensis</i>	TPH removal from petroleum contaminated soils	Enhanced oxidation of shorter chain alkanes	Soleimani et al. (2010)

Note: IAA: Indole acetic acid; ACC: 1-aminocyclopropane-1-carboxylic acid; TPH: Total petroleum hydrocarbon.



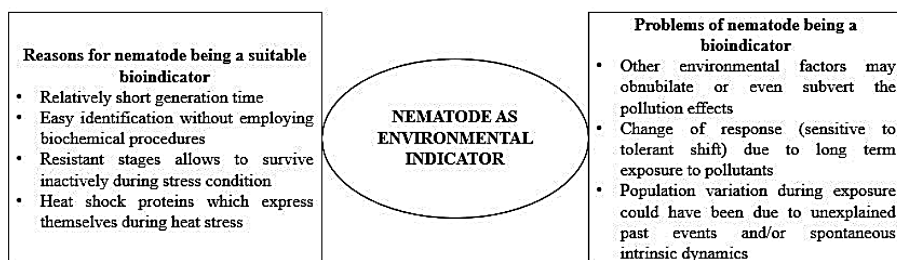
processes. Plant-endophyte synergism is important for plant development, growth, and fitness, as well as contaminated soil decontamination. The endophyte *Pseudomonas putida* PD1 was shown to enhance root and shoot development as well as protect plants against phenanthrene phytotoxicity in two distinct willow clones and a grass (Khan et al., 2014). The expression of degrading genes was found to be high in endophyte colonization that was successful (Jabeen et al., 2016).

Since plants do not procure their carbon from organic pollutants thus, they rely on the endophytes for their degradation. Endophytic bacteria create diverse enzymes to mineralize organic pollutants and reduce both phytotoxicity and evapotranspiration of volatile organic pollutants during phytoremediation of organic pollutants (Wang & Dai, 2011). For the first time, endophytic bacteria were used to clean up soil that had been contaminated by the organochlorine pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) (Germaine et al., 2006; Afzal, Khan, & Sessitsch, 2014). *Pseudomonas* sp. BF1-3, a root endophyte with the organophosphorus hydrolase gene *ophB*, can easily hydrolyze chlorpyrifos (Barman et al., 2014). *Burkholderia phytofirmans* PsJN, an endophyte, has glutathione-S-transferase (GST) genes that help it degrade and detoxify complex chemical substances (Mitter et al., 2013). Chelating agents, siderophores, biosurfactants, low molecular weight organic acids, and other detoxifying enzymes are all produced by certain endophytes and are all beneficial for eliminating organic pollutants from soils (Li et al., 2012). *Pestalotiopsis palmarum* BM-04, a powerful remediating endophytic fungus, can withstand high salinity and crude oil contamination (Naranjo-Briceño et al., 2013). PAH and total petroleum hydrocarbon (TPH) clearance were 80–84% and 64–72% in the plant rhizosphere, respectively, and TPH removal was higher in endophyte-infected plants than in non-infected plants (Soleimani et al., 2010; Nandy et al., 2020). *Phomopsis liquidambaris* could not only respond to phenanthrene pollution stress *in vitro*, but it could also mitigate the effects of phenanthrene accumulation in plants (Fu et al., 2018). Table 1.4 lists the endophytes involved in the detoxification of organic contaminants.

## 1.6 NEMATODES: AS INDICATORS AND AMELIORANTS OF HEAVY METAL CONTAMINATED SOILS

Nematodes are one of the most abundant multicellular animals on earth widely distributed in various soil habitats, with a total estimated population reaching to around  $4.4 \times 10^{20}$  (with a total biomass of approximately 0.3 gigatons) in the surface soil (van den Hoogen et al., 2019). Nematodes are found to play a

significant role in organic matter decomposition, nutrient cycling, enhance soil microbial activity and modify the composition of the microbial community structure (Ferris, Venette, & Scow, 2004; Xiao et al., 2010; Landesman, Treonis, & Dighton, 2011). They are ubiquitous and have successfully adapted to nearly every ecosystem of any climatic extreme. They are prevalent in a varied range of soils and aquatic systems, in heavily polluted soil, in deep-sea sediments and in decomposing plant and animal products. Conventional physical assessment of environment pollution provides an absolute value of pollutant concentration instead of its pathway and bioavailability. Therefore, biomonitor species could be employed as a feasible means to assess the bioavailability of pollutants and for risk assessment. Nematodes are quite sensitive to environmental factors and xenobiotic substances (organic and/or heavy metals) in soil and aquatic environment. Nematodes have several unique features *viz.* relatively short generation time, easy identification without employing biochemical procedures, resistant stages allowing them to survive inactively during stress condition and have heat shock proteins (HSPs) which express themselves during heat stress (Bongers & Ferris, 1999) (Figure 1.3). Thus, the use of nematodes as indicators of environmental stress caused by xenobiotic substance has caught the interest of researchers worldwide. However, there are several problems that could narrow the scope of use of nematode for environmental biomonitoring, the most important being the masking effect of other environmental factors on the actual effect of contaminants on nematode (Devi et al., 2021; Lal et al., 2020; Altaf et al., 2021).



**FIGURE 1.3** Pros and cons of use of nematode as a suitable environmental indicator.

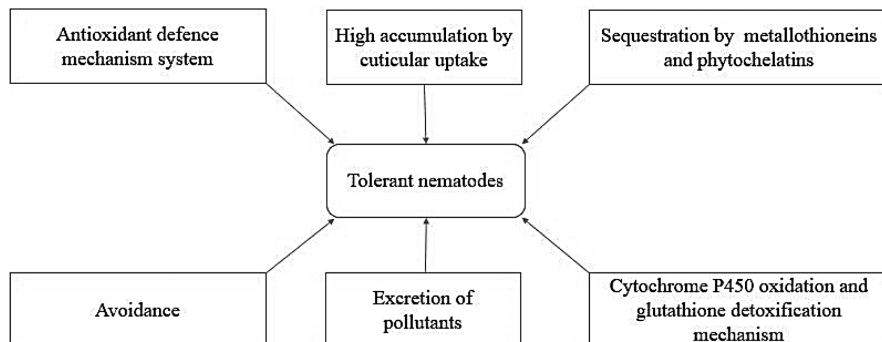
This could be avoided by minimizing the effect of external environmental factors other than the pollutants which could be facilitated by taking reference sites in a study which should be similar to contaminated localities (Sochová, Hofman, & Holoubek, 2006). Several studies have found that nematode community structure and diversity, not overall nematode

population, are the most sensitive features for detecting environmental stress (Yeates & Bongers, 1999). Plant parasitic nematodes, bacterial, and fungal feeders, predators, and omnivores all have various levels of susceptibility to soil pollutants. The eating habits and anatomy of the stoma determine the trophic groups (Gomes, Huang, & Cares, 2003; Sochová, Hofman, & Holoubek, 2006). Various ecological indices and characteristics are used to quantify soil disturbance levels and decomposition routes, as well as to monitor community changes in diversity and trophic structure (Table 1.5). Nematode community indices can also be used to characterize heavy metal's long-term impacts (Nagy et al., 2004).

Pollution levels (heavy metal or organic) in the environment have been shown to have a negative impact on soil ecology. Severe pollution causes a notable reduction in species variety and is generally straightforward to identify, but adequate environmental protection necessitates the identification of less severe consequences. Furthermore, any minor changes in community structure or population caused by pollution are frequently obscured by other external variables, making it difficult to track. Thus, to avoid this, pollution tolerant species of nematode could be used as indicators that detect the impact of pollutants but do not respond to changes in other environmental variables (Millward & Grant, 2000; Beeby, 2001). Nematodes have been shown to react distinctively to different pollutants. Some commonly found tolerance mechanisms towards organic and inorganic pollutants are outlined in Figure 1.4. Due to its extensive host range and tolerance to a variety of climatic situations, *Xiphinema vuittenezi*, a plant pathogenic nematode, has been used as a test organism in laboratory toxicity and heavy metal absorption investigations (Sávolly et al., 2016; Hrács et al., 2018). The greater resistance of *X. vuittenezi* to bulk ZnO was attributed by Hrács et al. (2018) to higher internal Zn levels in the species owing to cuticular absorption of Zn. *Panagrellus redivivus*, a free-living bacterivore nematode and r-strategist nematode, is also commonly employed as a test organism for aquatic and terrestrial toxicity investigations (Leitgib, Kálmán, & Gruiz, 2007). Nematodes can stop pharyngeal pumping and avoid ingesting hazardous chemicals by doing so (Jones, Peter, & Candido, 1999). The detoxification of organic xenobiotics and the discharge of metal ions from the cell are both aided by GSH and glutathione S-transferases (GST). GSH is a redox regulator and antioxidant that aids in the elimination of reactive oxygen species (ROS), particularly hydrogen peroxide ( $H_2O_2$ ) (van den Hoogen et al., 2019; Tiwari et al., 2021; Lal, Vengavasi, & Pandey, 2019). This is thought to be an essential nematode tolerance mechanism.

**TABLE 1.5** Various Ecological Indices to Study Community Changes of Nematode Under Stress

Parameters/Ecological Index	Calculation/Definition	Purpose	References
Colonizer–persister (cp) values	The cp scale corresponds to r–K strategy: cp 1 (colonizers, opportunists) are r-strategists, cp 5 (persisters) are K-strategists.	Categorization of nematode taxa	Bongers & Ferris (1999)
Maturity index (MI)	Weighted mean of the c-p values for all individual nematodes in a representative soil sample excluding plant parasite nematodes.	Monitor colonization and subsequent succession after disturbances, measure xenobiotic-induced stress, differentiate between tillage practices, aftereffects of liming an acid soil	Bongers & Ferris (1999)
Plant parasitic index (PPI)	Weighted mean of the c-p values for all individual plant parasite nematodes in a representative soil sample.		
Modified maturity index (mMI)	Weighted mean of the c–p values for all individual nematodes in a representative soil sample		
PPI/MI ratio	–	Assessment and monitoring of the status recovery of disturbed habitats.	Yeates & Bongers (1999)
Shannon-Weaver’s diversity index	$H' = -\sum p_i \log_2 p_i$ where; $p_i$ is $n/N$ , $n$ being the number of organisms of the $i^{\text{th}}$ species and $N$ the total number of organisms in the sample	Characterize nematode diversity under stress	Shannon & Grieve (1998)
Simpson’s diversity index	$Ds = 1 - \sum(p_i)^2$ where; $p_i$ is the percent of genus ‘i’ in the total abundance.	–	Hurlbert (1971)



**FIGURE 1.4** Physiological responses responsible for imparting tolerance in nematodes to polluted soils.

GST has a wide range of activities, but its primary role is as a GSH transferase, catalyzing the conjugation of electrophilic endobiotics and xenobiotics (or their metabolites) to GSH (Li et al., 2012). GSTs also have non-catalytic functions, such as serving as binding and carrier proteins, such as ligands for certain hazardous chemicals. These processes are required for cell survival and detoxification against reactive oxygen species (ROS) and toxic electrophiles. Metallothioneins can operate as radical scavengers and guard against metal toxicity by sequestering Zn, Cu, Cd, and Hg, among other metals (Klaassen et al., 2019). If metabolism and excretion do not keep up with intake rates, organic contaminants can build up in the tissue. In *Caenorhabditis elegans*, Haitzer et al. (2000) discovered pyrene buildup in lipid-rich body areas. Pollution avoidance, detoxification, and accumulation (organic)/sequestration (heavy metals) are all accompanied by a variety of more general strategies for surviving in stressful situations (Ekschmitt & Korthals, 2006). These bioindicators' responses to remediation tactics can be used to examine how soil remediation approaches alter hazardous metal bioavailability in the environment (Harrington et al., 2012).

## 1.7 CHALLENGES ASSOCIATED WITH MICROBE-ASSISTED BIOREMEDIATION

Microbial degradation, in its broadest definition, refers to the employment of microbes to break down, degrade, detoxify, or change contaminants in the environment. Microbe-assisted bioremediation is unquestionably a significantly more cost-effective and environmentally benign method of

dealing with the considerable accumulation of contaminants (both organic and inorganic) in soil and water systems. As per an estimation made by Blaylock et al. (1997), adoption of bioremediation technique for Pb polluted soil could help reduce the cost by 50–65% compared to the conventional methods. Literature is replete with case studies implying the use of microbes as a successful clean-up strategy for both organic and inorganic pollutants. Microbes alone or in association with plants have the ability to metabolize a wide range of pollutants, categorically diverse in nature and properties, either to obtain carbon and/or energy for growth and development or as co-substrates. Despite numerous advantages of microbe-assisted bioremediation, several limitations persist which often thwart the extrapolation of lab carried experiments to field level showing unparalleled results. One particular concern that persists is the long-term sustainability of the microbes in bioremediation process. Considering the alarming rate of accumulation of both organic and inorganic pollutants in the environment the microbial bioremediation process should be fastened so as to match the available commercial remediation processes.

Presence of more than one type of pollutants or a mixture of organic pollutants and heavy metal(loid)s could obstruct the normal metabolic functioning of the microbes. Therefore, the microbes need to be genetically manipulated so as to introduce the required feature in the GEMs to increase their endurance level to a wide array of pollutants without affecting its remediation efficacy. In addition, genetic modification should be such that the GEM should be able to modulate itself to the changing environmental conditions. If at all the need of GEM arises then its introduction should be backed up with thorough studies on its ecotoxicity, effect on existing biodiversity and growth kinetics. Microbial techniques are the most logical, long-term answer for remediation because the safe removal of large levels of contaminants is a concern matched by greater worldwide awareness of the environmental concerns caused by other approaches.

## **1.8 CONCLUSION AND FUTURE PROSPECTS**

Natural and human activities are causing an increase in organic and inorganic contaminants in the environment. In order to have a balanced and sustainable ecosystem, these toxins must be removed and/or stabilized from the environment. Despite the fact that there have been several targeted studies in this area, only a few strategies have been shown to be both safe and cost-effective.

Plants with high efficiency and rhizobacteria/genetically modified (GM) microorganisms are thought to be preferable to expensive physiochemical remediation approaches. The use of nematodes as an indication for stressed environments produced by heavy metal pollution, on the other hand, appears to be an added benefit in bioremediation systems. However, these biological approaches reveal slow microbial metabolic growth kinetics. Degradation of a complex combination of contaminants by a single species of microorganism is also reported to be a time-consuming process. As a result, finding microbial strains that can stimulate plant development while also degrading/stabilizing contaminants is important, and future research should concentrate on GM microorganisms for bioremediation and their mechanism of action in successfully degrading pollutants. In addition, for successful pollution remediation, the reaction model, degradation kinetics, and half-life period of the degraded pollutants must be evaluated.

## KEYWORDS

- **anthropogenic activities**
- **bioremediation**
- **environmental pollutants**
- **inorganic pollutants**
- **organic pollutants**
- **secondary pollution**

## REFERENCES

- Afzal, M., Qaiser, M. K., & Angela, S., (2014). Endophytic bacteria: Prospects and applications for the phytoremediation of organic pollutants. *Chemosphere*, 117(1), 232–242. Pergamon. doi: 10.1016/j.chemosphere.2014.06.078.
- Altaf, M. A., Rabia, S., Ming, X. R., Latif, U. K., Muhammad, M. A., Mohammad, S. J., Muhammad, A. N., et al., (2021). Protective mechanisms of melatonin against vanadium phytotoxicity in tomato seedlings: Insights into nutritional status, photosynthesis, root architecture system, and antioxidant machinery. *Journal of Plant Growth Regulation*, 1–17. Springer. doi: 10.1007/s00344-021-10513-0.
- Altaf, M. A., Rabia, S., Ming-Xun, R., Safina, N., Muhammad, M. A., Latif, U. K., Milan, K. L., et al., (2022). Melatonin mitigates cadmium toxicity by promoting root architecture

- and mineral homeostasis of tomato genotypes. *Journal of Soil Science and Plant Nutrition*, 1–17. Springer. doi: 10.1007/s42729-021-00720-9.
- Arora, N. K., (2018). Bioremediation: A green approach for restoration of polluted ecosystems. *Environmental Sustainability*, 1(4), 305–307. Springer. doi: 10.1007/s42398-018-00036-y.
- Babu, A. G., Patrick, J. S., Sudhakar, D., Ik Boo, J., & Byung, T. O., (2015). Potential use of *pseudomonas koreensis* AGB-1 in association with miscanthus sinensis to remediate heavy metal(loid)-contaminated mining site soil. *Journal of Environmental Management*, 151, 160–166. Academic Press. doi: 10.1016/j.jenvman.2014.12.045.
- Barman, D. N., Md Azizul, H., Shah Md, A. I., Han, D. Y., & Min, K. K., (2014). Cloning and expression of OphB gene encoding organophosphorus hydrolase from endophytic *Pseudomonas Sp.* BF1-3 degrades organophosphorus pesticide chlorpyrifos. *Ecotoxicology and Environmental Safety*, 108, 135–141. Academic Press. doi: 10.1016/j.ecoenv.2014.06.023.
- Barzanti, R., Francesca, O., Marco, B., Roberto, G., Francesca, G., Cristina, G., & Alessio, M., (2007). Isolation and characterization of endophytic bacteria from the nickel hyperaccumulator plant *Alyssum bertolonii*. *Microbial Ecology*, 53(2), 306–316. Springer. doi: 10.1007/s00248-006-9164-3.
- Beeby, A., (2001). “What do sentinels stand for?” *Environmental Pollution*, 112(2), 285–298. Elsevier. doi: 10.1016/S0269-7491(00)00038-5.
- Bhardwaj, D., Mohammad, W. A., Ranjan, K. S., & Narendra, T., (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, 13(1), 1–10. BioMed Central Ltd. doi: 10.1186/1475-2859-13-66.
- Bilal, S., Raheem, S., Abdul, L. K., Al-Harrassi, A., Chang, K. K., & In Jung, L., (2019). Phytohormones enabled endophytic *Penicillium funiculosum* LHL06 protects *glycine max* L. from synergistic toxicity of heavy metals by hormonal and stress-responsive proteins modulation. *Journal of Hazardous Materials*, 379, 120824. Elsevier. doi: 10.1016/j.jhazmat.2019.120824.
- Blaylock, M. J., David, E. S., Slavik, D., Olga, Z., Christopher, G., Yoram, K., et al., (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science and Technology*, 31(3), 860–865. American Chemical Society. doi: 10.1021/es960552a.
- Bongers, T., & Howard, F., (1999). Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology and Evolution*, 14(6), 224–228. Elsevier Current Trends. doi: 10.1016/S0169-5347(98)01583-3.
- Brown, N. L., Jivko, V. S., Stephen, P. K., & Jon, L. H., (2003). The MerR family of transcriptional regulators. *FEMS Microbiology Reviews*, 27(2, 3), 145–163. Oxford Academic. doi: 10.1016/S0168-6445(03)00051-2.
- Chinnusamy, V., Jian, K. Z., & Ramanjulu, S., (2010). Gene regulation during cold stress acclimation in plants. *Methods in Molecular Biology (Clifton, N.J.)*, 639, 39–55. Humana Press. doi: 10.1007/978-1-60761-702-0\_3.
- Chourasia, K. N., Sanket, J. M., Ashok, K., Dharmendra, K., Brajesh, S., Vinay, B., Awadhesh, K., et al., (2022). Salinity responses and tolerance mechanisms in underground vegetable crops: An integrative review. *Planta*. doi: <https://doi.org/10.1007/s00425-022-03845-y>.
- Costley, S. C., & Wallis, F. M., (1999). Effect of disk rotational speed on heavy metal accumulation by rotating biological contactor (RBC) biofilms. *Letters in Applied Microbiology*, 29(6), 401–405. John Wiley & Sons, Ltd. doi: 10.1046/j.1472-765X.1999.00661.x.



- Dary, M., Chamber-Pérez, M. A., Palomares, A. J., & Pajuelo, E., (2010). 'In situ' phytostabilization of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. *Journal of Hazardous Materials*, 177(1–3), 323–330. doi: 10.1016/j.jhazmat.2009.12.035.
- Deng, Z., Lixiang, C., Haiwei, H., Xinyu, J., Wenfeng, W., Yang, S., & Renduo, Z., (2011). Characterization of Cd- and Pb-resistant fungal endophyte *Mucor* Sp. CBRF59 isolated from rapeseed (*Brassica chinensis*) in a metal-contaminated soil. *Journal of Hazardous Materials*, 185(2, 3), 717–724. Elsevier. doi: 10.1016/j.jhazmat.2010.09.078.
- Devi, R., Biswaranjan, B., Md Basit, R., Vikas, M., Muhammad, A. A., Ravinder, K., Awadhesh, K., et al., (2021). An insight into microbes mediated heavy metal detoxification in plants: A review. *Journal of Soil Science and Plant Nutrition*, 41(4), 1–23. doi: 10.1007/S42729-021-00702-X.
- Dhanya, M. S., & Arun, K., (2020). Bioremediation: An eco-friendly cleanup strategy for polyaromatic hydrocarbons from petroleum industry waste. *Bioremediation of Industrial Waste for Environmental Safety* (pp. 399–436). Springer, Singapore. doi: 10.1007/978-981-13-1891-7\_18.
- Dupont, R. R., (1993). Fundamentals of bioventing applied to fuel contaminated sites. *Environmental Progress*, 12(1), 45–53. John Wiley & Sons, Ltd. doi: 10.1002/ep.670120109.
- Ekschmitt, K., & Gerard, W. K., (2006). Nematodes as sentinels of heavy metals and organic toxicants in the soil. *Journal of Nematology*, 38(1), 13–19. Society of Nematologists. /pmc/articles/PMC2586444/.
- Ferris, H., Venette, R. C., & Scow, K. M., (2004). Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralization function. *Applied Soil Ecology*, 25(1), 19–35. Elsevier. doi: 10.1016/j.apsoil.2003.07.001.
- Fu, W., Man, X., Kai, S., Liyan, H., Wei, C., Chuanchao, D., & Yong, J., (2018). Biodegradation of phenanthrene by endophytic fungus *Phomopsis liquidambari* in *Vitro* and *in Vivo*. *Chemosphere*, 203, 160–169. Pergamon. doi: 10.1016/j.chemosphere.2018.03.164.
- Fuller, W. H., (1977). *Movement of Selected Metals, Asbestos, and Cyanide in Soil: Applications to Waste Disposal Problems*. Municipal Environmental Research Laboratory, Office of Research.
- Gentry, T. J., Christopher, R., & Ian, L. P., (2004). New approaches for bioaugmentation as a remediation technology. *Critical Reviews in Environmental Science and Technology*, 34(5), 447–494. Taylor & Francis Group. doi: 10.1080/10643380490452362.
- Germaine, K. J., Xuemei, L., Guiomar, G. C., Jill, P. H., David, R., & David, N. D., (2006). Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. *FEMS Microbiology Ecology*, 57(2), 302–310. Oxford Academic. doi: 10.1111/j.1574-6941.2006.00121.x.
- Ghosh, M., & Singh, S. P., (2005). A review on phytoremediation of heavy metals and utilization of its byproducts. *Applied Ecology and Environmental Research*, 3(1), 1–18. doi: 10.15666/aecer/0301\_001018.
- Gomes, G. S., Shiou, P. H., & Juvenil, E. C., (2003). Nematode community, trophic structure and population fluctuation in soybean fields. *Fitopatologia Brasileira*, 28(3), 258–266. SciELO Brasil.
- Guimarães, A., Ana, C., Natalie, M. S., Gabriel, A. C., Marla, M., Thiago, P. L., Petzl-Erler, M. L., Ricardo, L. R. S., et al., (2021). Tracing the distribution of European lactase persistence genotypes along the Americas. *Frontiers in Genetics*, 12, 1282. Frontiers Media S.A. doi: 10.3389/fgene.2021.671079.

- Gulan, L., Biljana, M., Tijana, Z., Gordana, M., & Biljana, V., (2017). Persistent organic pollutants, heavy metals and radioactivity in the urban soil of Priština City, Kosovo and Metohija. *Chemosphere*, 171, 415–426. Elsevier.
- Guo, H., Shenglian, L., Liang, C., Xiao, X., Qiang, X., Wanzhi, W., Guangming, Z., et al., (2010). Bioremediation of heavy metals by growing hyperaccumulator endophytic bacterium *Bacillus* Sp. L14. *Bioresource Technology*, 101(22), 8599–8605. Elsevier. doi: 10.1016/j.biortech.2010.06.085.
- Haghollahi, A., Mohammad, H. F., & Mahin, S., (2016). The effect of soil type on the bioremediation of petroleum contaminated soils. *Journal of Environmental Management*, 180, 197–201. Elsevier.
- Harekrushna, S., & Das, C. K., (2012). A review on: Bioremediation. *International Journal of Research in Chemistry and Environment*, 2(1), 13–21.
- Harrington, A. J., Talene, A. Y., Sunny, R. S., Kim, A. C., & Guy, A. C., (2012). Functional analysis of VPS41-mediated neuroprotection in *Caenorhabditis elegans* and mammalian models of Parkinson's disease. *Journal of Neuroscience*, 32(6), 2142–2153. Soc Neuroscience.
- He, L., Huan, Z., Guangxia, L., Zhongmin, D., Philip, C. B., & Jianming, X., (2019). Remediation of heavy metal contaminated soils by biochar: Mechanisms, potential risks and applications in China. *Environmental Pollution*, 252, 846–855. Elsevier. doi: 10.1016/j.envpol.2019.05.151.
- Hooda, V., (2007). Phytoremediation of toxic metals from soil and wastewater. *Journal of Environmental Biology*, 28(2), 367. Citeseer.
- Hrács, K., Zoltán, S., Anikó, S., Lola, V. K., Ibolya, Z. P., Ákos, K., Gyula, Z., & Péter, N., (2018). Toxicity and uptake of nanoparticulate and bulk ZnO in nematodes with different life strategies. *Ecotoxicology*, 27(8), 1058–1068. Springer. doi: 10.1007/s10646-018-1959-8.
- Huang, D., Wenjing, X., Guangming, Z., Jia, W., Guomin, C., Chao, H., Chen, Z., et al., (2016). Immobilization of Cd in river sediments by sodium alginate modified nanoscale zero-valent iron: Impact on enzyme activities and microbial community diversity. *Water Research*, 106, 15–25. Pergamon. doi: 10.1016/j.watres.2016.09.050.
- Huang, G. H., Feng, C., Dong, W., Xue, W. Z., & Gu, C., (2010). biodiesel production by microalgal biotechnology. *Applied Energy*, 87(1), 38–46. Elsevier. doi: 10.1016/j.apenergy.2009.06.016.
- Huo, W., Chun, H. Z., Ya, C., Meng, P., Hui, Y., Lai, Q. L., & Qing, S. C., (2012). Paclobutrazol and plant-growth promoting bacterial endophyte *Pantoea* Sp. enhance copper tolerance of guinea grass (panicum maximum) in hydroponic culture. *Acta Physiologiae Plantarum*, 34(1), 139–150. Springer. doi: 10.1007/s11738-011-0812-y.
- Hurlbert, S. H., (1971). the nonconcept of species diversity: A critique and alternative parameters. *Ecology*, 52(4), 577–586. John Wiley & Sons, Ltd. doi: 10.2307/1934145.
- Hyman, M., & Ryan, D. R., (2001). *Groundwater and Soil Remediation*. American Society of Civil Engineers. doi: 10.1061/9780784404270.
- Jabeen, H., Samina, I., Fiaz, A., Muhammad, A., & Sadiqa, F., (2016). Enhanced remediation of chlorpyrifos by ryegrass (*Lolium multiflorum*) and a chlorpyrifos degrading bacterial endophyte *Mezorhizobium* Sp. HN3. *International Journal of Phytoremediation*, 18(2), 126–133. Taylor & Francis. doi: 10.1080/15226514.2015.1073666.
- Jaganathan, D., Karthikeyan, R., Gothandapani, S., Shilpha, J., & Gayatri, V., (2018). CRISPR for crop improvement: An update review. *Frontiers in Plant Science*. Frontiers media S.A. doi: 10.3389/fpls.2018.00985.

- Jariyal, M., Manish, Y., Nitin, K. S., Suman, Y., Iti, S., Swati, D., & Arti, T., (2020). Microbial remediation progress and future prospects. *Bioremediation of Pollutants*, 187–214. Elsevier. doi: 10.1016/b978-0-12-819025-8.00008-9.
- Jones, D., Peter, E., & Candido, M., (1999). feeding is inhibited by sublethal concentrations of toxicants and by heat stress in the nematode *Caenorhabditis elegans*: Relationship to the cellular stress response. *Journal Of Experimental Zoology*, 284, 147–157. doi: 10.1002/(SICI)1097-010X(19990701)284:2.
- Khan, F. I., Tahir, H., & Ramzi, H., (2004). An overview and analysis of site remediation technologies. *Journal of Environmental Management*, 71(2), 95–122. Academic Press. doi: 10.1016/j.jenvman.2004.02.003.
- Khan, N., & Asghari, B., (2016). Role of plant growth promoting rhizobacteria and ag-nano particle in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation. *International Journal of Phytoremediation*, 18(3), 211–221. Taylor & Francis. doi: 10.1080/15226514.2015.1064352.
- Khan, Z., & Sharon, D., (2011). Endophyte-assisted phytoremediation. *Plant Biol.*, 12, 97–105.
- Khan, Z., David, R., Trent, K., May, D. A., Raymond, Y., & Sharon, D., (2014). Degradation, phytoprotection and phytoremediation of phenanthrene by endophyte *Pseudomonas putida*, PD1. *Environmental Science & Technology*, 48(20), 12221–12228. ACS Publications.
- Klaassen, M. T., Peter, M. B., Chris, M., & Luisa, M. T., (2019). Multi-allelic QTL analysis of protein content in a bi-parental population of cultivated tetraploid potato. *Euphytica*, 215(2). doi: 10.1007/s10681-018-2331-z.
- Kloepper, J. W., (1978). Plant growth-promoting rhizobacteria on radishes. In: *Proc. of the 4<sup>th</sup> Internat. Conf. on Plant Pathogenic Bacter*, Station de Pathologie Vegetale et Phytobacteriologie (Vol. 2, pp. 879–882). INRA, Angers, France.
- Kumar, A., Bisht, B. S., Joshi, V. D., & Dhewa, T., (2011). Review on bioremediation of polluted environment: A management tool. *International Journal of Environmental Sciences*, 1(6), 1079–1093. Integrated Publishing Association.
- Kumar, A., Goutam, K. D., Madhusmita, B., Puja, A. P., Milan, K. L., Mirza, J. B., & Padmini, S., (2020). Effect of drought stress on resistant starch content and glycemic index of rice (*Oryza Sativa* L.). *Starch/Staerke*, 72(11, 12), 1900229. Wiley. doi: 10.1002/star.201900229.
- Kumar, A., Sarangadhar, N., Umakanta, N., Rameswar, P. S., Milan, K. L., Azharudheen, T. P., Sasmita, B., et al., (2021). A single nucleotide substitution in the SPDT transporter gene reduced phytic acid and increased mineral bioavailability from rice grain (*Oryza Sativa* L.). *Journal of Food Biochemistry*, 45(7), e13822. John Wiley & Sons, Ltd. doi: 10.1111/jfbc.13822.
- Kumar, D., Som, D., Pinky, R., Sushil, S. C., Milan, K. L., Rahul, K. T., Kumar, N. C., & Brajesh, S., (2021). Beneficial microorganisms in crop growth, soil health, and sustainable environmental management. *Plant-Microbial Interactions and Smart Agricultural Biotechnology*, 11–32. Boca Raton: CRC Press. doi: 10.1201/9781003213864-2.
- Kumar, V., & Gaurav, S., (2020). *Microbe-Assisted Phytoremediation of Environmental Pollutants: Recent Advances and Challenges*. AK Peters/CRC Press.
- Lal, M. K., Awadhesh, K., Ashok, K., Pinky, R., Augustine, O. O., Nitasha, T., Vandana, P., Asha, T., & Brajesh, S., (2020). Dietary fibers in potato. In: *Potato*, 37–50. Springer. doi: 10.1007/978-981-15-7662-1\_3.

- Lal, M. K., Awadhesh, K., Ashok, K., Rupak, J., Pinky, R., Dharmendra, K., Nitasha, T., & Brajesh, S., (2020). Minerals in potato. *Potato*, 87–112. Springer, Singapore. doi: 10.1007/978-981-15-7662-1\_6.
- Lal, M. K., Brajesh, S., Rahul, K. T., Sudhir, K., Gopalakrishnan, S., Kishore, G., Awadhesh, K., Vijay, P., & Madan, P. S., (2022). Interactive effect of retrogradation and addition of pulses, cooking oil on predicted glycemic index and resistant starch of potato. *Starch*, 74, 2100221.
- Lal, M. K., Krishnapriya, V., & Renu, P., (2019). Interactive effects of low phosphorus and elevated CO<sub>2</sub> on root exudation and nutrient uptake in wheat is modified under Sulphur nutrition. *Plant Physiology Reports*, 24(1), 63–73. Springer Nature. doi: 10.1007/s40502-019-0433-9.
- Lal, M. K., Rahul, K. T., Vijay, G., Vikas, M., Awadhesh, K., Madan, P. S., Vijay, P., et al., (2021). Physiological and molecular insights on wheat responses to heat stress. *Plant Cell Reports*, 1, 1–18. Springer. doi: 10.1007/s00299-021-02784-4.
- Landesman, W. J., Amy, M. T., & John, D., (2011). Effects of a one-year rainfall manipulation on soil nematode abundances and community composition. *Pedobiologia*, 54(2), 87–91. Elsevier.
- Leitgib, L., Judit, K., & Katalin, G., (2007). Comparison of bioassays by testing whole soil and their water extract from contaminated sites. *Chemosphere*, 66(3), 428–434. Elsevier.
- Li, Hai-Yan, Da-Qiao, W., Mi, S., & Zuo-Ping, Z., (2012). Endophytes and their role in phytoremediation. *Fungal Diversity*, 54(1), 11–18. Springer.
- Luo, Sheng-Lian, Liang, C., Jue-Liang, C., Xiao, X., Tao-Ying, X., Yong, W., Chan, R., et al., (2011). Analysis and characterization of cultivable heavy metal-resistant bacterial endophytes isolated from Cd-hyperaccumulator *Solanum nigrum* L. and their potential use for phytoremediation. *Chemosphere*, 85(7), 1130–1138. Elsevier.
- Mangal, V., Milan, K. L., Rahul, K. T., Muhammad, A. A., Salej, S., Dharmendra, K., Vinay, B., et al., (2022). Molecular insights into the role of reactive oxygen, nitrogen and Sulphur species in conferring salinity stress tolerance in plants. *Journal of Plant Growth Regulation*, 1–21. Springer. doi: 10.1007/s00344-022-10591-8.
- Masindi, V., & Khathutshelo, L. M., (2018). Environmental contamination by heavy metals. *Heavy Metals*. doi: 10.5772/intechopen.76082.
- Meena, O. P., Rakesh, S., Ashok, K. G., Kailash, C. G., Biswaranjan, B., Rani, S., Malu, R. Y., et al., (2022). Energy-carbon footprint vis-à-vis system productivity and profitability of diversified crop rotations in semi-arid plains of North-West India. *Journal of Soil Science and Plant Nutrition*, 1–16. Springer. doi: 10.1007/s42729-022-00791-2.
- Millward, R. N., & Alastair, G., (2000). Pollution-induced tolerance to copper of nematode communities in the severely contaminated restronguet creek and adjacent estuaries, Cornwall, United Kingdom. *Environmental Toxicology and Chemistry: An International Journal*, 19(2), 454–461. Wiley Online Library.
- Mishra, R. K., Naseer, M., & Nilanjan, R., (2016). Soil pollution: Causes, effects and control. *Van Sangyan*, 3(1), 1–14.
- Mishra, V., Antriksh, G., Parvinder, K., Simranjeet, S., Nasib, S., Praveen, G., & Joginder, S., (2016). Synergistic effects of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in bioremediation of iron contaminated soils. *International Journal of Phytoremediation*, 18(7), 697–703. Taylor & Francis.
- Mitter, B., Alexandra, P., Maria, W. S., Patrick, S. G. C., Hauberg-Lotte, L., Reinhold-Hurek, B., Jerzy, N., & Angela, S., (2013). Comparative genome analysis of *Burkholderia*

- phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. *Frontiers in Plant Science*, 4, 120. Frontiers.
- Mondal, M., Gopinath, H., Gunapati, O., Thingujam, I., & Onkar, N. T., (2019). Bioremediation of organic and inorganic pollutants using microalgae. In: *New and Future Developments in Microbial Biotechnology and Bioengineering*, 223–235. Elsevier.
- Moussa, H. R., & Sabah, M. A. A., (2017). Does exogenous application of melatonin ameliorate boron toxicity in spinach plants? *International Journal of Vegetable Science*, 23(3), 233–245. Taylor and Francis Inc. doi: 10.1080/19315260.2016.1243184.
- Nagy, P., Gabor, B., Tom, B., Imre, K., Miklos, F., & Istvan, K., (2004). Effects of microelements on soil nematode assemblages seven years after contaminating an agricultural field. *Science of the Total Environment*, 320(2, 3), 131–143. Elsevier.
- Nandy, S., Tuyelee, D., Champa, K. T., Devendra, K. P., Abhijit, D., & Puja, R., (2020). Fungal endophytes: Futuristic tool in recent research area of phytoremediation. *South African Journal of Botany*, 134, 285–295. Elsevier.
- Nannipieri, P., Judith, A., Maria, T. C., Loretta, L., Giacomo, P., Giancarlo, R., & Federico, V., (2007). Microbial diversity and microbial activity in the rhizosphere. *Ciencia Del Suelo*, 25(1), 89–97. SciELO Argentina.
- Naranjo-Briceño, L., Beatriz, P., Mayamaru, G., Jhonny, R. D., Ángela De, S., Ysvic, I., Meralys, G., et al., (2013). Potential role of oxidative exoenzymes of the extremophilic fungus *Pestalotiopsis palmarum* BM-04 in biotransformation of extra-heavy crude oil. *Microbial Biotechnology*, 6(6), 720–730. Wiley Online Library.
- Norris, R. D., (1993). *Handbook of Bioremediation*. CRC press.
- Norris, R. D., (1995). *In-Situ Bioremediation of Ground Water and Geological Material: A Review of Technologies*. DIANE Publishing.
- Oladipo, O. G., Olusegun, O. A., Akinyemi, O., Cornelius, C. B., & Mark, S. M., (2018). Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. *Brazilian Journal of Microbiology*, 49(1), 29–37. Elsevier Editora Ltda. doi: 10.1016/j.bjm.2017.06.003.
- Pietro-Souza, W., Felipe De, C. P., Ivani, S. M., Fernando, F. F. S., Ailton, J. T., Cátia Nunes Da, C., James, F. W., et al., (2020). Mercury resistance and bioremediation mediated by endophytic fungi. *Chemosphere*, 240, 124874. Pergamon. doi: 10.1016/j.chemosphere.2019.124874.
- Poulsen, L. V., (1999). Microbial biofilm in food processing. *LWT - Food Science and Technology*, 32(6), 321–326. Academic Press. doi: 10.1006/fstl.1999.0561.
- Poulsen, T. G., & Kai, B., (2010). Organic micropollutant degradation in sewage sludge during composting under thermophilic conditions. *Environmental Science and Technology*, 44(13), 5086–5091. American Chemical Society. doi: 10.1021/es9038243.
- Pramanik, K., Soumik, M., Anumita, S., & Tushar, K. M., (2018). Alleviation of phytotoxic effects of cadmium on rice seedlings by cadmium resistant PGPR strain *Enterobacter aerogenes* MCC 3092. *Journal of Hazardous Materials*, 351, 317–329. Elsevier. doi: 10.1016/j.jhazmat.2018.03.009.
- Rodríguez, A. A., Santiago, J. M., Ana, B. M., & Oscar, A. R., (2009). Polyamine oxidase activity contributes to sustain maize leaf elongation under saline stress. *Journal of Experimental Botany*, 60(15), 4249–4262. Oxford Academic. doi: 10.1093/jxb/erp256.
- Sávoly, Z., Krisztina, H., Bernhard, P., Christina, S., Gyula, Z., & Péter, I. N., (2016). Uptake and toxicity of nano-ZnO in the plant-feeding nematode, *Xiphinema vuittenzei*: The role

- of dissolved zinc and nanoparticle-specific effects. *Environmental Science and Pollution Research*, 23(10), 9669–9678. Springer. doi: 10.1007/s11356-015-5983-4.
- Sayler, G. S., & Steven, R., (2000). Field applications of genetically engineered microorganisms for bioremediation processes. *Current Opinion in Biotechnology*, 11(3), 286–289. doi: 10.1016/S0958-1669(00)00097-5.
- Schaechter, M., (2009). *Encyclopedia of Microbiology*. Academic Press.
- Schiewer, S., & Bohumil, V., (2014). Biosorption processes for heavy metal removal. *Environmental Microbe-Metal Interactions*, 329–362. Wiley Online Library. doi: 10.1128/9781555818098.ch14.
- Scott, C., Cameron, B., Matthew, J. T., Gunjan, P., Vinko, M., Nigel, F., Clint, B., et al., (2011). Free-enzyme bioremediation of pesticides: A case study for the enzymatic remediation of organophosphorus insecticide residues. *ACS Symposium Series*, 1075, 155–174. American Chemical Society. doi: 10.1021/bk-2011-1075.ch011.
- Shannon, M. C., & Grieve, C. M., (1998). Tolerance of vegetable crops to salinity. *Scientia Horticulturae*. Elsevier. doi: 10.1016/S0304-4238(98)00189-7.
- Shilev, S., Mladen, N., Ventsislava, V., & Anna, A., (2007). Composting of food and agricultural wastes. *Utilization of By-Products and Treatment of Waste in the Food Industry*, 283–301. Springer, Boston, MA. doi: 10.1007/978-0-387-35766-9\_15.
- Shim, H., & Shang, T. Y., (1999). Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by a coculture of *Pseudomonas Putida* and *Pseudomonas fluorescens* immobilized in a fibrous-bed bioreactor. *Journal of Biotechnology*, 67(2, 3), 99–112. Elsevier. doi: 10.1016/S0168-1656(98)00166-7.
- Sobariu, D. L., Daniela, I. T. F., Mariana, D., Lucian, V. P., Raluca, M. H., Elena, N. D., Silvia, C., et al., (2017). Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. *New Biotechnology*, 39, 125–134. Elsevier. doi: 10.1016/j.nbt.2016.09.002.
- Sochová, I., Jakub, H., & Ivan, H., (2006). Using nematodes in soil ecotoxicology. *Environment International*, 32(3), 374–383. Pergamon. doi: 10.1016/j.envint.2005.08.031.
- Soleimani, M., Majid, A., Mohammad, A. H., Farshid, N., Mohammad, R. S., & Jan, H. C., (2010). Phytoremediation of an aged petroleum contaminated soil using endophyte infected and non-infected grasses. *Chemosphere*, 81(9), 1084–1090. Pergamon. doi: 10.1016/j.chemosphere.2010.09.034.
- Sun, L. N., Yan, F. Z., Lin, Y. H., Zhao, J. C., Qing, Y. W., Meng, Q., & Xia, F. S., (2010). Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-tolerant plant species on copper mine wasteland. *Bioresource Technology*, 101(2), 501–509. Elsevier. doi: 10.1016/j.biortech.2009.08.011.
- Tiwari, R. K., Bishnu, M. B., Shanmugam, V., Milan, K. L., Ravinder, K., Sanjeev, S., Kailash, C. N., et al., (2021). First report of dry rot of potato caused by *Fusarium proliferatum* in India. *Journal of Plant Diseases and Protection*, 1–7. Springer. doi: 10.1007/s41348-021-00556-6.
- Tiwari, R. K., Bishnu, M. B., Shanmugam, V., Milan, K. L., Ravinder, K., Sanjeev, S., Vinod, et al., (2021). Impact of *Fusarium* dry rot on physicochemical attributes of potato tubers during postharvest storage. *Postharvest Biology and Technology*, 181, 111638. Elsevier. doi: 10.1016/j.postharvbio.2021.111638.
- Tiwari, R. K., Milan, K. L., Ravinder, K., Vikas, M., Muhammad, A. A., Sanjeev, S., Brajesh, S., & Manoj, K., (2021). Insight into melatonin-mediated response and signaling in the regulation of plant defense under biotic stress. *Plant Molecular Biology*. Springer. doi: 10.1007/s11103-021-01202-3.

- Trotta, A., Falaschi, P., Cornara, L., Minganti, V., Fusconi, A., Drava, G., & Berta, G., (2006). Arbuscular mycorrhizae increase the arsenic translocation factor in the as hyperaccumulating fern *Pteris vittata* L. *Chemosphere*, 65(1), 74–81. Pergamon. doi: 10.1016/j.chemosphere.2006.02.048.
- UNEP, (2015). *Sustainable Consumption and Production – Handbook for Policymakers*. Global Edition; Nairobi: United Nations Environment Program.
- Van, D. H., Johan, S. G., Devin, R., Howard, F., Walter, T., David, A. W., Ron, G. M. De. G., et al., (2019). Soil nematode abundance and functional group composition at a global scale. *Nature*, 572(7768), 194–198. Nature Publishing Group. doi: 10.1038/s41586-019-1418-6.
- Van, D. J., Lloyd, T., Chhetry, S., Liou, R., & Peck, J., (2002). *Remediation Technologies Screening Matrix and Reference Guide-Version 4.0*. US Army Environmental Center, Report/# SFIM-AEC-ET-CR-97053, Aberdeen Proving Ground, MD.
- Vidali, M., (2001). Bioremediation - an overview. *Pure and Applied Chemistry*, 73(7), 1163–1172. De Gruyter.
- Visioli, G., D'Egidio, S., Teofilo, V., Monica, M., & Anna, M. S., (2014). Culturable endophytic bacteria enhance Ni translocation in the hyperaccumulator *Noccaea caerulea*. *Chemosphere*, 117(1), 538–544. Pergamon. doi: 10.1016/j.chemosphere.2014.09.014.
- Wang, W., Zujun, D., Hongming, T., & Lixiang, C., (2013). Effects of Cd, Pb, Zn, Cu-resistant endophytic *Enterobacter* sp. CBSB1 and *Rhodotorula*Sp. CBSB79 on the growth and phytoextraction of *Brassica* plants in multimetal contaminated soils. *International Journal of Phytoremediation*, 15(5), 488–497. Taylor & Francis Group. doi: 10.1080/15226514.2012.716101.
- Wang, Y., & Chuan-Chao, D., (2011). Endophytes: A potential resource for biosynthesis, biotransformation, and biodegradation. *Annals of Microbiology*, 61(2), 207–215. BioMed Central.
- Weyens, N., Sarah, C., Joke, D., Lee, N., Daniel, V. D. L., Robert, C., & Jaco, V., (2010). Endophytic bacteria improve phytoremediation of Ni and TCE co-contamination. *Environmental Pollution*, 158(7), 2422–2427. Elsevier. doi: 10.1016/j.envpol.2010.04.004.
- Williams, J., (2006). *Bioremediation of Contaminated Soils: A Comparison of In Situ and Ex Situ Techniques*. Recuperado de/Paper/Bioremediation-of-Contaminated-Soils-% 3A-A-Comparison-Williams/4c6afc722040e0d4807a744b7f89a5e7b9dac97f, 2001.
- Wu, B., Zirui, W., Yuxing, Z., Yuanming, G., Ying, W., Jiang, Y., & Heng, X., (2019). The performance of biochar-microbe multiple biochemical material on bioremediation and soil micro-ecology in the cadmium aged soil. *Science of the Total Environment*, 686, 719–728. Elsevier. doi: 10.1016/j.scitotenv.2019.06.041.
- Wu, Y., & Ji, C., (2013). Investigating the effects of point source and nonpoint source pollution on the water quality of the East River (Dongjiang) in South China. *Ecological Indicators*, 32, 294–304. Elsevier. doi: 10.1016/j.ecolind.2013.04.002.
- Xiao, A. W., Li, Z., Li, W. C., & Ye, Z. H., (2020). The effect of plant growth-promoting rhizobacteria (PGPR) on arsenic accumulation and the growth of rice plants (*Oryza sativa* L.). *Chemosphere*, 242, 125136. Pergamon. doi: 10.1016/j.chemosphere.2019.125136.
- Xiao, H., Bryan, G., Xiaoyun, C., Manqiang, L., Jiaguo, J., Feng, H., & Huixin, L., (2010). Influence of bacterial-feeding nematodes on nitrification and the ammonia-oxidizing bacteria (AOB) community composition. *Applied Soil Ecology*, 45(3), 131–137. Elsevier. doi: 10.1016/j.apsoil.2010.03.011.
- Yamamoto, T., Ikue, G., Osamu, K., Kazuaki, M., Eiji, A., & Osamu, M., (2008). Phytoremediation of shallow organically enriched marine sediments using benthic

- microalgae. *Marine Pollution Bulletin*, 57(1–5), 108–115. Pergamon. doi: 10.1016/j.marpolbul.2007.10.006.
- Yeates, G. W., & Bongers, T., (1999). Nematode diversity in agroecosystems. *Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes*, 113–135. Elsevier. doi: 10.1016/b978-0-444-50019-9.50010-8.
- Yin, K., Qiaoning, W., Min, L., & Lingxin, C., (2019). Microorganism remediation strategies towards heavy metals. *Chemical Engineering Journal*, 360, 1553–1563. Elsevier. doi: 10.1016/j.cej.2018.10.226.
- Yousaf, S., Muhammad, A., Thomas, G. R., Carrie, L. B., & Angela, S., (2011). Hydrocarbon degradation, plant colonization and gene expression of alkane degradation genes by endophytic *Enterobacter ludwigii* strains. *Environmental Pollution*, 159(10), 2675–2683. Elsevier. doi: 10.1016/j.envpol.2011.05.031.
- Yuan, M., Huaidong, H., Li, X., Ting, Z., Hui, L., Shubin, L., Peiyan, D., Zhihong, Y., & Yuanxiao, J., (2014). Enhancement of Cd phytoextraction by two *Amaranthus* species with endophytic *Rahnella* Sp. JN27. *Chemosphere*, 103, 99–104. Pergamon. doi: 10.1016/j.chemosphere.2013.11.040.
- Zahoor, M., Muhammad, I., Hazir, R., Muhammad, Q., Sahib, G. A., Muhammad, Q., & Anwar, H., (2017). Alleviation of heavy metal toxicity and phytostimulation of *Brassica campestris* L. by endophytic *Mucor* Sp. MHR-7. *Ecotoxicology and Environmental Safety*, 142, 139–149. Academic Press. doi: 10.1016/j.ecoenv.2017.04.005.
- Zeraatkar, A. K., Hossein, A., Ahmad, F. T., Navid, R. M., & Mark, P. M., (2016). Potential use of algae for heavy metal bioremediation, a critical review. *Journal of Environmental Management*, 181, 817–831. Academic Press. doi: 10.1016/j.jenvman.2016.06.059.
- Zhuang, X., Jian, C., Hojae, S., & Zhihui, B., (2007). New advances in plant growth-promoting rhizobacteria for bioremediation. *Environment International*, 33(3), 406–413. Pergamon. doi: 10.1016/j.envint.2006.12.005.





# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

## CHAPTER 2

---

# Rhizospheric Microbial Inoculation in Developing Stress Tolerance

SUCHISMITA PRUSTY,<sup>1</sup> MONALISHA DASMOHAPATRA,<sup>1</sup>  
SAGAR MAITRA,<sup>2</sup> and RANJAN KUMAR SAHOO<sup>1\*</sup>

<sup>1</sup>*Department of Biotechnology, Centurion University of Technology and Management, Paralakhemundi – 761211, Odisha, India,*

<sup>2</sup>*Department of Agronomy and Agroforestry, Centurion University of Technology and Management, Paralakhemundi – 761211, Odisha, India*

*\*Corresponding author. E-mail: ranjan.sahoo@cutm.ac.in*

---

### ABSTRACT

Biotic and abiotic stresses are key restrictions in agricultural yield, food grade, and universal food stability. Numerous characteristics are influenced by stress, including physiological, biochemical, and molecular plant conditions. Soil fertility and environmental contamination have both declined as a result of the usage of inorganic fertilizers and pesticides in agricultural practices. As a result, it is critical to construct more secure and long-term agricultural development methods. Plant growth-stimulating microorganisms (PGPM) and mycorrhizal fungi are used to boost plant growth in these situations. It offers a cost-effective and environmentally friendly way to protect plants from stress. Plant growth can be aided by PGPM, which regulates plant hormones, improves nutrient acquisition, generates siderophores, and improves antioxidant techniques and systems. Acquired systemic resistance (ASR) along with mediated systemic resistance (MSR) are useful in combating biotic stress (ISR). In stressed conditions, arbuscular mycorrhiza (AM) improves nutrient and water transportation while also increasing stress

toleration. This interrelation between the plant and the microbe is significant for viable agrarian along with industrial purposes since it relies on biological activities and restores traditional agricultural practices. As an environmental engineer, you can use microbes to help solve environmental stress problems. As a result, feeding the world's population with available resources while reducing environmental effects is a feasible and future technology. We have endeavored to investigate beneficial microorganisms that can withstand abiotic and biotic stress, as well as their mechanisms of action, in order to boost long-term agricultural production.

## 2.1 INTRODUCTION

Abiotic and biotic stress are constantly affecting our agroecosystem, affecting crop fecundity, soil condition, and prolificacy. The presence of innumerable stress-causing factors has an adverse effect on growth and productivity of crops. The biotic as well as abiotic tensions are the two kinds of stressors. Abiotic and biotic strain are causing 50% and 30% of losses in farm output, respectively. These stresses can be occurred naturally or produced by humans. Temperature, dryness, salinity, and heavy metal stress are the most significant abiotic stressors. The impacts of stress on the biochemistry along with morphological, physiological as well as on the gene regulation of the plant are numerous. In relation to climate and temperature change, scarcity of water, brininess, and heavy metal pollution are key stressors. Abiotic stress variables also have an impact on biotic stress, lowering crop output. The loss of soil microbial diversity, soil fertility, and competition for nutrient resources are the main effects of these pressures (Chodak et al., 2015). Plant-linked microbial communities, like fungal mycorrhiza and plant growth-promoting bacteria (PGPB), are the only viable alternatives, as they aid plant flourishing and maturation under various biotic as well as abiotic conditions. Plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi are two efficient microorganisms that can help improve and enhance viable agricultural as well as environs reliability. Plant-linked microorganisms are divided into three categories based on their effects on plants: helpful, harmful, and neutral. PGPR refers to a bacterial group including *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Serratia*, along with *Variovorax* that support plant blooming and development in both normal and stressful conditions. Drought, salt, and heavy metal stress are all too much for most plant growth-promoting microorganisms (PGPM) and *Arbuscular mycorrhizae* (AM). As a result, developing biofertilizers that are appropriate in such conditions is a difficult undertaking

for both farmers and scientists. Plant fitness and health are maintained by the PGPM and mycorrhizae in biotic as well as abiotic tensile environments (Vimal et al., 2017). Upcoming task will be production of biofertilizers that can be used alleviation of the stresses. Some of them laced with the ability to withstand these pressures and encourage plant flourishing and maturation. These tensile-tolerant microorganisms have a unique way of overcoming adversity and consolidating plants. However, in regard to investigate plant related microbes' interactions for influencing plant flourishing and disease defiance in feasible agriculture, new methodologies are required (Finkel et al., 2017). In the matter of biotic and abiotic tensity, the plant-correlated serviceable microorganisms improve the proficiency of their maturation and flourishing. In this chapter, we sought to investigate the advantageous effects of distress-tolerating microorganisms as well as their mechanisms of action in order to improve the sustainability of agrarian production.

## **2.2 PGPM ASSISTING STRESS TOLERANCE**

The implementation of strain-tolerant AM fungi as well as PGPM to plants may improve their thriving and surviving rate under harsh conditions (Nadeem et al., 2014). Microbes exploited indirect as well as direct ways to support plant thriving and maturation amid stressful situations. Microorganisms use a variety of biochemical and molecular pathways to stimulate maturation and development. Inoculation with PGPM, as an example, promotes plant development by maintaining balance in hormone and nutritional state, creating plant growth regulators, and prompting phytopathogen resistivity (Spence & Bais, 2015). PGPM produces compounds that lower pathogen populations in the plant's environment. Production of siderophore in the rhizosphere by these bacteria, for example, restricted the availability of iron to specific pathogens, causing a reduction in their development (Zoch et al., 2016). In addition, they help plants growth by nitrogen fixation from the air, solubilizing phosphate, along with generating plant hormones (Ahmad et al., 2011). Additional strategies that assist the plant adapt with the unfavorable environment include nutrient mobilization, exopolysaccharide formation, rhizobitoxine production, and so on (Vardharajula et al., 2011). Ethylene production is inhibited by the rhizobitoxine, which helps plants to grow and develop in stressful situations (Kumar et al., 2009). Moreover, important enzymes such as ACC-deaminase, chitinase, and glucanase might have the capability to improve the thriving and developmental rate of plant subjected to stress conditions (Farooq et al., 2009). Furthermore, the sigma factors present in few bacteria allow them to modify the expression of gene in

unfavorable conditions in order to counteract detrimental effects (Gupta et al., 2013). Aside from PGPM, another crucial aspect of growth and development is the reciprocal action of fungi with the roots present in the higher plants. AM is the most frequent type of mycorrhizae found in agricultural fields. These fungi are crucial players in the nutrient cycle, absorption, and translocation.

### 2.3 METHOD AND MODE OF DROUGHT STRESS TOLERANCE

The microorganisms that are tolerant to drought conditions have the potential to improve plant survivability and development when there is a lack of water. To survive in low water potential environments, microorganisms have transformed, adapted, and/or developed a mode for tolerance (Table 2.1). They can gather osmolytes and create exo-polysaccharides, and they can form thick walls or go inactive (EPS). These plant-associated microorganisms have a variety of ways for dealing with the obstructive effects of drought on both the plants and soil. They give nutrients and superior environmental conditions for ongoing plant growth, regardless of water content. Beneficial microorganisms that colonize the rhizosphere enhance plant growth and development in a variety of ways, both directly and indirectly: (i) synthesis of phytohormones such as cytokinins, indole-3-acetic acid (IAA) as well as abscisic acid (ABA); (ii) exopolysaccharides from bacteria; (iii) ACC deaminase; and (iv) elevated systemic toleration are all possible mechanisms.

Plant-produced phytohormones play an important role in maturation and survivability (Farooq et al., 2009; Porcel et al., 2014). Furthermore, PGPR has the potential to generate plant-related hormones that promote plant division and maturity in stressful situations. During drought stress, IAA, a highly active auxin, governs differentiation in the vascular tissues along with adventitious and/or lateral differentiation of roots, division of cells, and growth of shoot (Goswami et al., 2015). ABA is a key growth-related regulator in drought-tolerant plants. As PGPR is introduced into a seed or plant, the concentration of ABA rises, regulating the physiology of the plant to resist drought stress. Drought strain is alleviated by ABA through modulating the drought-related genes transcription and hydraulic conductivity through roots (Jiang et al., 2013). *Azospirillum brasilense* recovers *Arabidopsis thaliana*'s drought response mostly through increasing levels of ABA (Cohen et al., 2015) (Table 2.1). During times of stress, 1-aminocyclopropane-1-carboxylate (ACC) acts as an instant antecedent of the ethylene. Hydrolyzation of ACC is done by ACC deaminase from the bacteria into ammonia and alpha-ketobutyrate (Bal

**TABLE 2.1** Microorganisms for Tolerance to Drought Stress

Name of Microorganism	Plants	Mechanism	References
<i>Azospirillum lipoferum</i>	Maize	Increase the amount of soluble sugar, free amino acids, and proline content. Root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight are all affected.	Grover et al. (2021)
<i>Bacillus</i> spp.	Maize	Proline, carbohydrates, and free amino acids accumulate more, and electrolyte leakage is reduced. It reduces the antioxidant enzyme's activity (catalase, glutathione peroxidase)	Abdelaal et al. (2021)
<i>Pseudomonas putida</i>	Soybean	The level of abscisic acid and salicylic acid decreases and the content of jasmonic acid increases.	Skz et al. (2018)
<i>Bacillus amyloliquefaciens</i>	Wheat	Transcript level-mediated homeostasis increases.	Safdarian et al. (2019)
<i>Pseudomonas putida</i>	<i>Cicer arietinum</i> L.	Accumulation of osmolyte, scavenging of ROS ability increases.	Tiwari et al. (2016)
<i>Azospirillum</i> sp.	Lettuce	Ascorbic acid, chlorophyll content and aerial biomass, increases.	Fascigleone et al. (2015)
<i>Azospirillum brasilense</i>	Wheat	Crop yield and proline level increased. Simultaneously decreased stomatal conductance, and relative soil water content.	Hernaández-Esquivel et al. (2020)
<i>Trichoderma</i>	Rice	Delay response against drought stress	Khadka & Uphoff (2019)
<i>Pseudomonas libanensis</i> and <i>Pseudomonas reactans</i>	<i>Brassica oxyrrhina</i>	Increased pigment content, plant growth and decreased proline and malondialdehyde content in leaves.	Ma et al. (2016)

et al., 2013). Drought tensile tolerance and PGPR improve biomass, potential of water, and reduce loss of water in stressed maize plants. These inoculants lessen the antioxidant action while increasing sugar, proline, and free amino acid synthesis in plants (Vardharajula et al., 2011).

## 2.4 MANAGING SALINITY STRESS

Farmers and agricultural scientists face a difficult problem in determining soil salinity. The cumulation of harmful sodium and chlorine ions inside the soil, as well as nutritional imbalances, have a significant impact on plant growth and microbial activity. Inoculating PGP microorganisms and endophytic microorganisms resulted in reduction of harmful salt effect on many plants. Plant growth under salinity stress can be aided by PGP microorganisms through a variety of direct and indirect ways. Furthermore, the biofilm generated by the PGPB under saline tensity is useful in reducing the negative consequences (Kasim et al., 2016). In a salty situation, *Azospirillum* inoculated lettuce seed demonstrated greater germination rate as well as advancement in vegetative maturation than the control (Barassi et al., 2006). In another survey, inoculating salt-resistant and salt susceptible chili pepper with the growth-promoting bacteria *Pseudomonas stutzeri* reduced the deleterious effects of brininess soil (Bacilio et al., 2016). Other species of microorganisms tend to reduce the action of biofilm formation in response to salinity stress when tested on grains of barley (Kasim et al., 2016). Reports suggested that in some plants, great improvements in salinity toleration is noticed when co-inoculation of AM fungi is done with salt-resistant bacteria. For example, when the salt tensile maize plants are inoculated with *R. intraradices* and *Massilia* sp. RK4, they together improved the colonization of arbuscular mycorrhizal fungi (AMF) in the root resulting in accumulation of the nutrients. These microbial as well as fungal interactions have a substantial impact on maize plant salinity tolerance (Krishnamoorthy et al., 2016).

### 2.4.1 MECHANISM OF SALINITY STRESS TOLERANCE

The stimulation of growth under stress conditions is aided by the diversity of salinity stress-resistant microorganisms (Table 2.2). Production of phytohormone (e.g., auxin, cytokinin, ethylene, and gibberellins), fixation of nitrogen, nutrients mobilization, and production of the siderophore are examples of direct methods (Hayat et al., 2010). All of them are laced with a unique mechanism of action as well as mode of operation. These actions

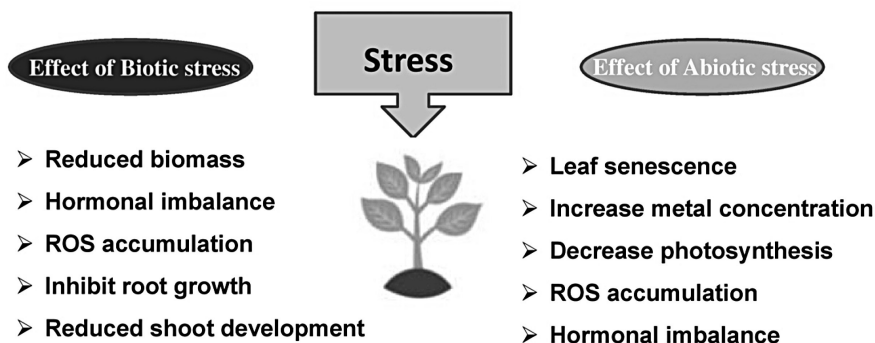
**TABLE 2.2** Microorganisms for Tolerance to Salinity Stress

Name of Microorganism	Plants	Mechanism	References
<i>Azospirillum</i>	Lettuce seeds	Promoted higher ascorbic acid content, higher biomass and lower the browning intensity	Fasciglione et al. (2015)
<i>Hartmannibacter diazotrophicus</i> E19	Barley ( <i>Hordeum vulgare</i> L.)	Increased ACC-deaminase activity, root, and shoot dry weight and also lower ethylene content	Suarez et al. (2015)
<i>Bacillus amyloliquefaciens</i> NBRISN13 (SN13)	Rice	Differences in transcription level in at least 14 genes	Chauhan et al. (2019)
<i>Pseudomonas</i> sp., <i>Serratia</i> sp.	Wheat	Reduce ethylene level, reduce ACC deaminase activity, and enhance root length, shoot height, and grain productivity.	Vimal et al. (2019)
<i>Azospirillum</i> sp.	Wheat	Increased grain yield and shoot dry weight. Plants accumulate proline and soluble sugars to maintain osmotic pressure	Elakhdar et al. (2019)
<i>Rhizobium</i> and <i>Pseudomonas</i>	Mung bean ( <i>Vigna radiate</i> )	Increases yield of mung bean, increases ACC-deaminase activity for growth and nodulation under salinity stress conditions	Ahmad et al. (2014)
<i>Acinetobacter</i> spp. and <i>Pseudomonas</i> sp.	Barley and oats	Promote IAA production and lower ACC deaminase for better growth and yield.	Chang et al. (2014)
<i>Brachybacterium saurashtrense</i> (JG06)	Groundnut ( <i>Arachis hypogaea</i> L.)	Higher phosphorus, calcium, and nitrogen. Higher K <sup>+</sup> /Na <sup>+</sup> ratio in shoot and root.	Shrivastava & Kumar (2015)



result in an elevation in the length of root, its surface area along with number of roots thereby increasing the uptake of nutrients (Egamberdieva & Kucharkova, 2007).

Reduction in recurrence of plant pathogens causing diseases is a primary indirect factor. Rhizobacteria that colonize the roots create ACC deaminase, which helps in the conversion of ammonia and alpha ketobutyrate from ACC, lowering ethylene. Under stress, rhizobitoxine enzyme is known to inhibit the synthesis of ethylene synthesis, thereby promoting the nodulations. In an attempt to reduce the salt tensity, the PGPB collects osmolytes in the cytoplasm, thereby creating a counteract to the osmotic stress along with keeping a check on the turgor pressure of the cell and the plant development. Salinity resistance of plant is achieved when the EPS binds to the cations, which explains its unavailability in plants in stress (Vardharajula et al., 2011). Co-inoculation with the strains of PGPR for example *Pseudomonas*, *Rhizobium*, etc., can help plants flourish in saline soil by overcoming these negative effects (Bano & Fatima, 2009). PGPR features were found in two rhizospheric bacteria isolated from saline soil, *Bacillus pumilus* and *Bacillus subtilis*, including IAA synthesis, hydrogen cyanide (HCN) and ammonia generation, solubilization of phosphates, and salt tensile resistance (Damo-daran et al., 2013) (Figure 2.1).



**FIGURE 2.1** Effect of different biotic and abiotic stresses on plants.

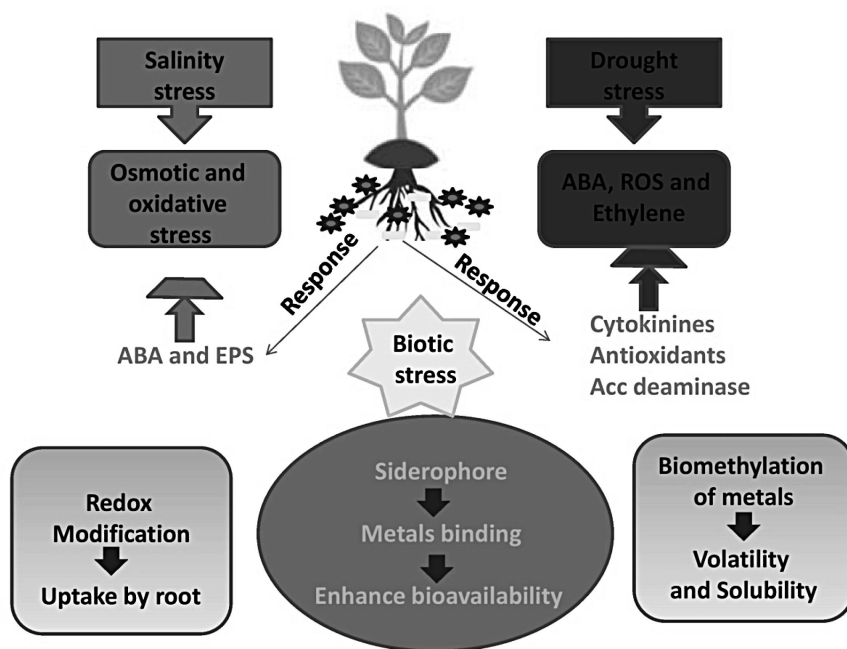
## 2.5 MYCORRHIZAL FUNGUS IN SALINITY AND DROUGHT STRESS TOLERANCE

The tolerance to salinity and drought distress is aided by plant-associated fungus. Drought stress is alleviated by AM symbiosis, which changes hormone physiology and plant physiology. It also boosts the effectiveness of

photosystem II and photosynthetic products when there is a water shortage (Ruiz-Lozano et al., 2016). Level of strigolactone rises in lettuce and tomato plants to alleviate drought tensivity by symbiosis development while improving drought resistance (Ruiz-Lozano et al., 2016). AM fungus has also been identified being a key element in reducing the salinity of soil. Inoculation with AM have shown to improve plant development in salt-stress conditions. In a saline environment, they prevent the absorption of sodium or chlorine by citrus plants (Navarro et al., 2014). As a result, inoculation with PGPR and various microorganisms can be used to help salt-sensitive crops cope with salinity stress. Co-inoculation of PGM microorganisms and plant-related fungi may improve the effectiveness of drought and salinity stress tolerance.

## 2.6 MICROBES AGAINST HEAVY METAL STRESS

Plant-related microbes, for example, firmicutes, rhizobacteria, mycorrhiza, etc., as well as heavy metal tolerant microorganisms, have the capability to enhance the growth and maturation of plants being in metal stress conditions. Efflux, metal impermeability, sequestration of EPS, volatilization, enzymatic detoxification, and complexation of metals are all methods involving these bacteria. Furthermore, these bacteria linked with plants stimulate plant growth and development by reducing ethylene levels, producing plant growth regulators, including IAA and deaminase ACC, as well as suppressing diseases (Glick, 2010) (Figure 2.2). Fixation of nitrogen, nutrient mobilization, siderophores, as well as solubilization of phosphate, among other things, help plants grow and remove heavy metals (Verma et al., 2013; Ahmad et al., 2011). Heavy metals have been removed using both live and non-living microbial biomass. Bioaccumulation of microbes is a powerful way to remove heavy metals from contaminated soil. Firmicutes, Actinobacteria along with Proteobacteria have been found to eliminate larger concentrations of lead, manganese, and Arsenic from soil polluted by metals (Zhang et al., 2015). According to Fatnassi et al. (2015), copper (Cu) concentrations above 1 mM harmed *Vicia faba* plant growth, but when infected with rhizobia and PGPR, the impacts were mitigated. AM fungi reduce malonaldehyde and  $H_2O_2$ , minimizing the adverse effects of stress caused due to deposition of cadmium (Hashem et al., 2016). Jing et al. (2014) in his research have shown that different species of *Enterobacter* and species of *Klebsiella* are efficient metal tolerant by creating plant growth chemicals when removing lead, cadmium, and zinc metal from polluted soil (Table 2.1).



**FIGURE 2.2** Mechanism of rhizosphere microbes to minimize the various biotic and abiotic stresses.

## 2.7 MICROBES IN TOLERANCE TO BIOTIC STRESS

Plant-microbe interactions are critical for appropriate growth and development in natural settings. They are vital for nutrition mobilization and pathogen defense (Shoebitz et al., 2009). Under abiotic stress, biological management of soil-transmitted illnesses, replacing the chemically produced agents, have a substantial impact on crop productivity. Different elicitors are released when microorganisms interact with plants, causing physio-biochemical changes in the flora distribution. For several months, these modifications result in disease resistance in the plant. An essential mechanism for biotic stress tolerance is the production of reactive oxygen species (ROS) and oxidative burst (Miller et al., 2010). The systemic acquired resistance along with induced systematic resistance (ISR) are two types of defensive reaction mechanisms induced by bacteria (SAR). Non-morbific root correlated plant's growth-boosting microorganisms may increase the ISR, whereas SAR includes changes in gene expression at the molecular level and is linked to pathogenesis associated/related (PR) proteins. Both ISR As well as

SAR have diverse gene induction and expression patterns that are dependent on the eliciting and regulatory pathways (Nawrocka & Maolepsza, 2013). Under biotic stress, PGPM induce SAR, that includes the assemblage of PR proteins and SA, whereas ISR depends on pathways associated with the regulation of jasmonate and ethylene (Salas-Marina et al., 2011; Bari & Jones, 2009). The NOS (nitrogen-oxygen species) as well as ROS (reactive oxygen species) have a significant impact on the generation of SA, JA, and ET, and they create a complicated networks that modulates pathogens (Bari & Jones, 2009; Choudhary & Johri, 2009). The ethylene and regulatory factors play a crucial while the PR genes are expressing themselves.

## **2.8 CONCLUSION**

Plant growth characteristics, productivity, and survivability are all affected by various kinds of abiotic and biotic stressors. Those crops and plants that can substitute their physio-biological properties because of the exhibition of salinity, drought, heat, cold, and alkalinity tolerant proteins can withstand stress conditions. Crop output, quality of food, and universal food security are all hampered by these pressures. Imbalance in hormones, nutrient mobilization, toxicity of ions, and disease vulnerability are all continuing to wreak havoc on maturation and survivability of the plant in the current climate. The only other option for dealing with plant stressors is by the development of microbiological contrivances and procedures for soil-plant-microbe interaction.

## **KEYWORDS**

- **abiotic stress**
- **drought**
- **heavy metal stress**
- **microbial inoculation**
- **salinity**
- **tolerance**

## REFERENCES

- Abdelaal, K., AlKahtani, M., Attia, K., Hafez, Y., Király, L., & Künstler, A., (2021). The role of plant growth-promoting bacteria in alleviating the adverse effects of drought on plants. *Biology (Basel)*, 10, 520. doi: 10.3390/biology10060520.
- Ahmad, M., Zahir, Z. A., Asghar, H. N., & Asghar, M., (2011). Inducing salt tolerance in mung bean through co-inoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. *Can. J. Microbiol.*, 57, 578–589.
- Ahmad, M., Zahir, Z., Nadeem, S., Nazli, F., Jamil, M., & Jamshaid, M., (2014). Physiological response of mung bean to *Rhizobium* and *Pseudomonas* based biofertilizers under salinity stress. *Pak. J. Agric. Sci.*, 51, 555–562.
- Bacilio, M., Moreno, M., & Bashan, Y., (2016). Mitigation of negative effects of progressive soil salinity gradients by application of humic acids and inoculation with *Pseudomonas stutzeri* in a salt-tolerant and a salt-susceptible pepper. *Appl. Soil Ecol.*, 107, 394–404.
- Bal, H. B., Nayak, L., Das, S., & Adhya, T., (2013). Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. *Plant Soil*, 366, 93–105.
- Bano, A., & Fatima, M., (2009). Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. *Biol. Fert. Soil*, 45, 405–413.
- Barassi, C. A., Ayrault, G., Creus, C. M., Sueldo, R. J., & Sobrero, M. T., (2006). Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce. *Sci. Hort.*, 109, 8–14.
- Bari, R., & Jones, J. D., (2009). Role of plant hormones in plant defense responses. *Plant Mol. Biol.*, 69, 473–488.
- Chang, P., Gerhardt, K. E., Huang, X., Yu, X., Glick, B. R., Gerwing, P. D., & Greenberg, B. M., (2014). Plant growth-promoting bacteria facilitate the growth of barley and oats in salt-impacted soil: Implications for phytoremediation of saline soils, *Int. J. Phytoremediation*, 16(11), 1133–1147. doi: 10.1080/15226514.2013.821447.
- Chauhan, P. S., Lata, C., Tiwari, S., Chauhan, A. S., Mishra, S. K., Agrawal, L., Chakrabarty, D., & Nautiyal, C. S., (2019). Transcriptional alterations reveal *Bacillus amyloliquefaciens*-rice cooperation under salt stress. *Sci. Rep.*, 9, 11912. <https://doi.org/10.1038/s41598-019-48309-8>.
- Chodak, M., Gołębiowski, M., Morawska-Płoskonka, J., Kuduk, K., & Niklińska, M., (2015). Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. *Ann. Microbiol.*, 65, 1627–1637.
- Cohen, A. C., Bottini, R., Pontin, M., Berli, F. J., Moreno, D., Boccanlandro, H., Travaglia, C. N., & Piccoli, P. N., (2015). *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. *Physiol. Plant.*, 153, 79–90.
- Damodaran, T., Sah, V., Rai, R. B., Sharma, D. K., Mishra, V. K., Jha, S. K., & Kannan, R., (2013). Isolation of salt tolerant endophytic and rhizospheric bacteria by natural selection and screening for promising plant growth-promoting rhizobacteria (PGPR) and growth vigor in tomato under sodic environment. *Afr. J. Microbiol. Res.*, 7, 5082–5089.
- Egamberdieva, D., Jabbarova, D., & Hashem, A., (2015). *Pseudomonas* induces salinity tolerance in cotton (*Gossypium hirsutum*) and resistance to *Fusarium* root rot through the modulation of indole-3-acetic acid. *Saudi J. Soil. Sci.*, 22, 773–779.

- Elakhdar, I., Elshikh, M., Allam, N., Kamal, F., & Staehelin, C., (2019). Evaluation of salt-tolerant *Azospirillum* spp. and its role in improvement of wheat growth parameter. *EBSS.*, 3, 15–17. 10.21608/jenvbs.2019.16428.1069.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A., (2009). Plant drought stress: Effects, mechanisms, and management. *Sustain. Agric.*, 153–188.
- Fasciglione, G., Casanovasa, E. M., Quillehauquya, V., Yommi, A. K., Goñi, M. G., Rourab, S. I., & Barassia, C. A., (2015). *Azospirillum* inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. *Sci. Hortic.*, 195, 154–162.
- Finkel, O. M., Castrillo, G., Paredes, S. H., González, I. S., & Dangl, J. L., (2017). Understanding and exploiting plant beneficial microbes. *Plant Biol.*, 38, 155–163.
- Glick, B. R., (2010). Using soil bacteria to facilitate phytoremediation. *Biotechnol. Adv.*, 28, 367–374.
- Goswami, D., Thakker, J. N., & Dhandhukia, P. C., (2015). Simultaneous detection and quantification of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) produced by rhizobacteria from l-tryptophan (Trp) using HPTLC. *J. Microbiol. Method.*, 110, 7–14.
- Grover, M., Bodhankar, S., Sharma, A., Sharma, P., Singh, J., & Nain, L., (2021). PGPR mediated alterations in root traits: Way toward sustainable crop production. *Front. Sust. Food Syst.*, 4, 287–298.
- Gupta, G., Panwar, J., & Jha, P. N., (2013). Natural occurrence of *Pseudomonas aeruginosa*: A dominant cultivable diazotrophic endophytic bacterium colonizing *Pennisetum glaucum* (L.) R. Br. *Appl. Soil Ecol.*, 64, 252–261.
- Hashem, A., Abd\_Allah, E. F., Alqarawi, A. A., Huqail, A. A., Egamberdieva, D., & Wirth, S., (2016). Alleviation of cadmium stress in *Solanum lycopersicum* L. by *Arbuscular mycorrhizal* fungi via induction of acquired systemic tolerance. *Saudi J. Biol. Sci.*, 23, 272–281.
- Hayat, R., Amara, U., Khalid, R., & Ahmed, I., (2010). Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann. Microbiol.*, 60, 579–598.
- Hernaández-Esquivel, A. A., Castro-Mercado, E., Valencia-Cantero, E., Alexandre, G., & García-Pineda, E., (2020). Application of *Azospirillum brasilense* lipopolysaccharides to promote early wheat plant growth and analysis of related biochemical responses. *Front. Sustain. Food Syst.*, 4, 579976. doi: 10.3389/fsufs.2020.579976.
- Jiang, S., Zhang, D., Wang, L., Pan, J., Liu, Y., Kong, X., Zhou, Y., & Li, D., (2013). A maize calcium-dependent protein kinase gene, ZmCPK4, positively regulated abscisic acid signaling and enhanced drought stress tolerance in transgenic *Arabidopsis*. *Plant Physiol. Biochem.*, 71, 112–120.
- Kasim, W. A., Gaafara, R. M., Abou-Alib, R. M., Omar, M. N., & Hewait, H. M., (2016). Effect of biofilm-forming plant growth promoting rhizobacteria on salinity tolerance in barley. *Ann. Agric. Sci.*, 61, 217–227.
- Khadka, R. B., & Uphoff, N., (2019). Effects of *Trichoderma* seedling treatment with system of rice intensification management and with conventional management of transplanted rice. *Peer J.*, 7, e5877. doi: 10.7717/peerj.5877.
- Krishnamoorthy, R., Kim, K., Subramanian, P., Senthilkumar, M., Anandham, R., & Sa, T., (2016). Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhance the tolerance of maize to salinity in coastal reclamation soil. *Agric. Ecosyst. Environ.*, 231, 233–239.

- Kumar, K. V., Srivastava, S., Singh, N., & Behl, H. M., (2009). The role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*. *J. Hazard Matter.*, 170, 51–57.
- Ma, Y., Rajkumar, M., Zhang, C., & Freitas, H., (2016). Inoculation of *Brassica oxyrrhina* with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. *J. Hazard Mater.*, 320, 36–44. doi: 10.1016/j.jhazmat.2016.08.009.
- Miller, G., Suzuki, N., Ciftci-Yilmaz, S., & Mittler, R., (2010). Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ.*, 33, 453–467.
- Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A., & Ashraf, M., (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.*, 32, 429–448.
- Navarro, J. M., Pérez-Tornero, O., & Morte, A., (2014). Alleviation of salt stress in citrus seedlings inoculated with arbuscular mycorrhizal fungi depends on the rootstock salt tolerance. *J. Plant Physiol.*, 171, 76–85.
- Nawrocka, J., & Małolepsza, U., (2013). Diversity in plant systemic resistance induced by *Trichoderma*. *Biol. Control.*, 67, 149–156.
- Ruiz-Lozano, J. M., Aroca, R., Zamarreño, A. M., Molina, S., Jiménez, B. A., Porcel, R., García-Mina, J. M., et al., (2016). Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. *Plant Cell Environ.*, 39, 441–452.
- Safdarian, M., Askari, H., Shariati, J., & Nematzadeh, G., (2019). Transcriptional responses of wheat roots inoculated with *Arthrobacter nitroguajacolicus* to salt stress. *Sci. Rep.*, 9, 1792. <https://doi.org/10.1038/s41598-018-38398-2>.
- Salas-Marina, M. A., Silva-Flores, M. A., Uresti-Rivera, E. E., Longoria, E. C., Estrella, A. H., & Flores, S. C., (2011). Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *Eur. J. Plant Pathol.*, 131, 15–26.
- Shoebitz, M., Ribaudo, C. M., Pardo, M. A., Cantore, M. L., Ciampi, L., & Curá, J. A., (2009). Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perenne* rhizosphere. *Soil Biol. Biochem.*, 41, 1768–1774.
- Shrivastava, P., & Kumar, R., (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation, *Saudi J. Biol. Sci.*, 22, 123–131.
- SkZ, A., Vardharajula, S., & Vurukonda, S. S. K. P., (2018). Transcriptomic profiling of maize (*Zea mays* L.) seedlings in response to *Pseudomonas putida* strain FBKV2 inoculation under drought stress. *Ann. Microbiol.*, 68, 331–349.
- Spence, C., & Bais, H., (2015). The role of plant growth regulators as chemical signals in plant-microbe interactions: A double edged sword. *Curr. Opin. Plant Biol.*, 27, 52–58.
- Suarez, C., Cardinale, M., Ratering, S., Steffens, D., Jung, S., Zapata, A., Geissler-Plaum, R., & Schnell, S., (2015). Plant growth-promoting effects of *Hartmannibacter diazotrophicus* on summer barley (*Hordeum vulgare* L.) under salt stress. *Appl. Soil Ecol.*, 95, 23–30. 10.1016/j.apsoil.2015.04.017.
- Tiwari, S., Lata, C., Chauhan, P. S., & Nautiyal, C. S., (2016). *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiol. Bioc.*, 99, 108–117.

- Vardharajula, S., Ali, S. Z., Grover, M., Reddy, G., & Bandi, V., (2011). Drought-tolerant plant growth promoting *Bacillus* spp.: Effect on growth osmolytes, and antioxidant status of maize under drought stress. *J. Plant Interactions*, 6, 1–14.
- Verma, J., Yadav, J., Tiwari, K. N., & Kumar, A., (2013). Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. *Ecol. Eng.*, 51, 282–286.
- Vimal, S. R., Gupta, J., & Singh, J. S., (2018). Effect of salt tolerant *Bacillus* sp. and *Pseudomonas* sp. on wheat (*Triticum aestivum* L.) growth under soil salinity: A comparative study. *Microbiol. Res.*, 9, 26–32. <https://doi.org/10.4081/mr.2018.7462>.
- Vimal, S. R., Singh, J. S., Arora, N., & Singh, S., (2017). Soil-plant-microbe interactions in stressed agriculture management: A review. *Pedosphere*, 27, 177–192.
- Zhang, J., Wang, L. H., Yang, J. C., Liu, H., & Dai, J. L., (2015). Health risk to residents and stimulation to inherent bacteria of various heavy metals in soil. *Sci. Tot. Environ.*, 508, 29–36.
- Zoch, M., Thiem, D., Kopciuch, R. G., & Hryniewicz, K., (2013). Synthesis of siderophores by plant-associated metallotolerant bacteria under exposure to Cd<sup>2+</sup>. *Chemosphere*, 156, 312–325.





# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

## CHAPTER 3

---

# Role of Endophytes, Plant Growth Promoting Rhizobacteria, and Arbuscular Mycorrhizal Fungi in Stress Tolerance

ANURON BANERJEE,<sup>1</sup> NIRMALYA CHAKRABORTY,<sup>2</sup>  
KRISHNENDU ACHARYA,<sup>2</sup> and NILANJAN CHAKRABORTY<sup>1\*</sup>

*<sup>1</sup>Department of Botany, Scottish Church College, Kolkata – 700006,  
West Bengal, India*

*<sup>2</sup>Molecular and Applied Mycology and Plant Pathology Laboratory,  
Department of Botany, University of Calcutta, Kolkata – 700019,  
West Bengal, India*

*\*Corresponding author. E-mail: nilanjanchak85@gmail.com*

---

### ABSTRACT

Stress is the condition which limits plants growth and exerts adverse effects on physiology as plants prioritize resistance overgrowth. Continuous climatic change and increasing environmental pollution introduces different abiotic and biotic stress in the plants which causes huge economic loss and a threat to plant biodiversity. In order to survive in stress condition plants, incorporate different physical, physiological, and molecular strategies that mainly include phytohormonal regulation and stress-related gene expressions. In this particular, it was found that plants in association with endophytes, plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) exhibited better potential to mitigate stress conditions. Endophytes,

PGPR, and AMF are symbionts, which promotes growth and induce tolerance in plants against different biotic and abiotic stresses through the regulation of different phytohormones, antioxidant enzymes, secondary metabolites, volatile compounds, stress-related genes, and a number of signaling molecules are activated by their interaction. PGPR and endophytic microbes stimulate the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase which lower ethylene levels and promotes growth in stress condition. During stress period, association of PGPR, AMF, and endophytes elevate the levels antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Likewise, during biotic stress situation, when pathogens attacks plants, these symbionts activate different signaling molecules including cyclin dependent protein kinase (CDK), mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) which results in hypersensitive responses, rapid cell death and induces PAMP-triggered immunity (PTI). In this chapter, we discussed about how endophytes, PGPR, and AMF protects plants from different biotic and abiotic stresses, which include drought, salinity, cold, flood, and heavy metal stresses. Also enlightened different mechanism through which endophytes, PGPR, and AMF confers tolerance and growth in plants during adverse situations.

### **3.1 INTRODUCTION**

The normal growth and development of the plant is supported by the different environmental factors, mainly the climatic condition and the soil profile. Soil is a dynamic and complex structure which is greatly influenced by the presence of a variety of stressors. The rapid climate change exhibited the severity of different stress condition which affects the crop production and currently it is a major problem of sustainable agriculture. These stresses can be categorized into biotic such as attack of different pathogens and pests and abiotic which mainly includes adverse climatic conditions like salinity, drought, cold, flooding, heavy metal, and heat stress. However, abiotic stress is considered as the main reason for yield losses, but biotic stressors also cause significant effects to plants. Plants may cope with one or a combination of these stresses. To deal with these stress conditions plants have evolved different protective strategies both at cellular and molecular levels some of which are phytohormonal regulation, production of antioxidant enzymes, changing of architecture of plants root, transcriptional control, signal transduction, etc.

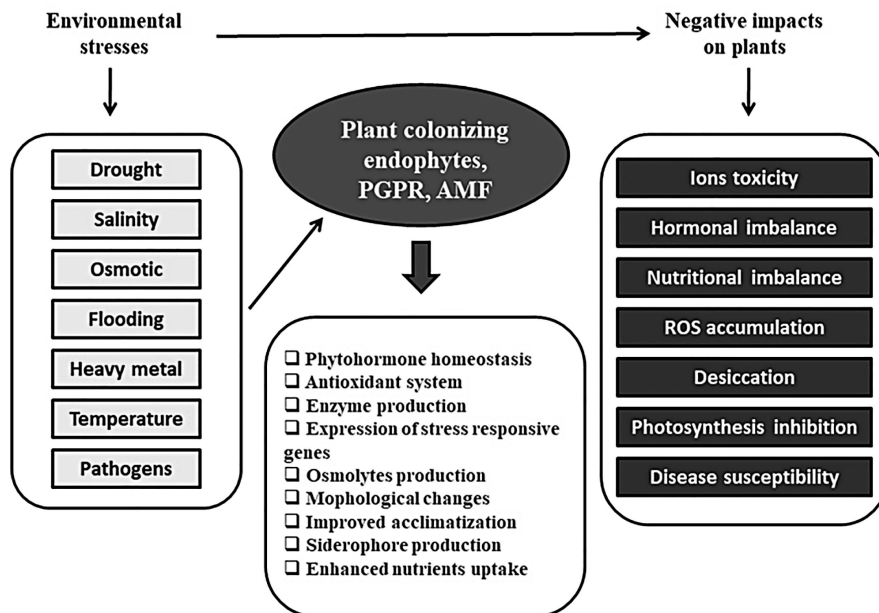
(Nadeem et al., 2013). In this particular, it was found that microbes that are associated with plants through the symbiotic relationship help to mitigate different stress conditions and promotes growth. Plant microbes' association is a very common incident and found in a plenty number of different plant species. Plant-microbes interactions induced tolerance in plant and also help to modulate plant response to adverse environmental conditions (Massad et al., 2012). Different beneficial bacteria, fungi, algae, protozoa, and actinomycetes that promote plants growth can be categorized into endophytes, PGPR and arbuscular mycorrhizal fungi (AMF). Not only in the stress condition but also in the normal condition, inoculation of plant roots with these microbes provides better plant growth and development. These microorganisms mainly harbor in the plant root and the rhizospheric area in the soil. The rhizosphere is the soil area that lies near the plant root supports maximum microbial activities since root exudates provides nutrients for their growth and metabolism (Ilanguaran & Smith, 2017).

Endophytes are symbiotic microorganisms that reside in the healthy plants without causing any significant harm and disease. Therefore, endophytic organisms are beneficial microbes that may be fungi, bacteria, or viruses (Lata et al., 2018). In 1866, De Bary first time coined the term endophytes for all the microbes that reside inside the healthy plant that do not develop any symptoms or disease (Verma et al., 2021). Geological time scale analysis has suggested that plant-endophytes interaction has originated from the time of origin of the first group of higher land plants and currently all the plants show plant-endophytes interaction. In recent time plant and endophytes interactions gained a special position as it could induce tolerance in plants against environmental stressors and also helps in inhibiting different pathogenic infections (Wiewiora et al., 2015). Endophytes can survive various adverse environmental conditions like nutrient deficit, drought, chilling conditions, etc. Fungal endophytes give protections to the photosynthetic apparatus in plants from heat and high light intensity and also induce the amount of photosynthetically active pigments (Rozpadek et al., 2015). Some of the fungal and bacterial endophytes help in sequestration of metals resulting in heavy metal tolerance in plants. Endophytes positively regulate the acclimatization process in plants under adverse climatic conditions and induce expression of stress responsive genes that are critical for the plant survival. Endophytes-mediated stress tolerance includes several direct and indirect mechanisms which includes providing enhanced nitrogen fixing abilities, production of osmolytes, phytohormones, Reactive Oxygen Species (ROS) scavenging enzymes, induced expression of defensive genes,

siderophore production and anti-pathogenic metabolites production (Zhang et al., 2019; Verma et al., 2021).

Among different microorganisms colonize in the plant roots, rhizospheric bacteria are the most common organisms that promote plant growth and exhibited antagonistic properties to different pathogens. Beneficial bacteria living in the plant root includes both symbiotic and free-living bacteria. This growth promoting free-living bacteria are known as plant growth promoting rhizobacteria or PGPR (Majeed et al., 2015). Beneficial bacteria belonging from different genera like *Bacillus*, *Azotobacter*, *Pseudomonas*, *Azospirillum*, *Klebsiella*, and *Enterobacter* are important PGPR that enhance plant growth and development under stress condition (Glick et al., 2007). Salinity is one of the severe threats to plant growth as it deteriorates the soil fertility and also induce osmotic stress. It was found that wheat species inoculated with a halophilic bacterium *Serratia* sp. improved salt tolerance in a Salt Lake (Singh & Jha, 2016). Beside this, PGPR also inhibit the disease cycle of several plant pathogens by exhibiting antagonistic mechanisms. There are some mechanisms through which PGPR enhance stress tolerance in plant which include production of enzymes like ACC-deaminase and chitinase, phytohormones, siderophore, inhibition of ethylene and induction of systemic resistance (Berg et al., 2009; Hyat et al., 2010).

Besides bacteria, fungal microorganisms occupied a great portion of the soil rhizosphere that supports plant growth and development. AMF found to form symbiotic relationship with 90% of plant and currently it is very difficult to find plants without having mycorrhizal colonization. Interaction between AMF and plants is considered as the most primitive symbiotic relationship that was first discovered (Diagne et al., 2020). AMF help in the uptake of different nutrients like phosphorus and nitrogen under stress condition and improved plant growth. AMF increases the root surface area that enables excess absorption of water and nutrients. Moreover, fungal hyphae can penetrate small pores of the soil, which contributes to more nutrient uptake and water absorption. Mycorrhizal fungi also help in the mobilization of those ions in the plant which are generally not found during abiotic stress condition (Allen, 2011; Faber et al., 1991). AMF not only promote plant growth but also it could induce tolerance against drought, salinity, temperature, and heavy metal stresses. An overall mechanism of how these symbiotic microorganisms provides tolerance to plants under stress condition is presented in Figure 3.1.



**FIGURE 3.1** Different environmental stress and the mechanisms through which rhizosphere microorganisms induced stress tolerance in plants.

### 3.2 PLANT AND DIFFERENT ENVIRONMENTAL STRESSES

Plants may be subjected to different environmental stresses through different abiotic and biotic factors. Stress can be defined as any adverse environmental condition which limits plants growth and development. In plant stress is caused by the principal abiotic factors like heat, salinity, cold, water logging, drought, heavy metals, nutrients deficit, intense light and also by biotic factors including pathogens and different pests (Lichtenthaler, 1998; Wahid et al., 2007). Abiotic stressors are the main challenge in the agricultural sector as it is responsible for the main yield losses every year worldwide. The scarcity of available water in the soil is responsible for different stress conditions like drought and salinity. Application of fertilizer along with irrigation and low rainfall greatly influenced salinity stress. It was reported that about 64% of the total arable land area of the world is under drought condition and 6% area is under salinity stress (Cramer et al., 2011). Drought is the most destructive stress condition that exhibit effects both at morphological and cellular level. One of the main effects of drought stress is reduced germination of seedlings (Nezhadahmadi et al., 2013). In addition to this, drought stress also inhibits photosynthetic efficiency of plants by decreasing leaf expansion along

with the accumulation of different ROS (Lata et al., 2018). Water deficit condition also affects several morphological and physiological changes like reduced root growth, delaying flowering and fruiting, reduced seed numbers and retard plant growth (Xu et al., 2016). Salt stress is another significant stressor which is characterized by high salt concentration in the soil that limits plant growth. Saline condition induces ion toxicity, reduced uptake of phosphorus and nitrogen, decreased membrane function, suppressed cellular metabolism, and accumulates ROS (Kumar, 2013). Rapid climate changes contribute to the global warming which also influences plant growth and development. High temperature rising has a direct detrimental effect on plants. Heat stress negatively impact protein stability, metabolism, RNA, and cytoskeleton structures (Theocharis et al., 2012). High temperature reduced seed germination and results in impaired photosynthetic machinery. In an experiment, it was observed that a short exposure of high temperature might cause fatal collapse of cellular organization and could cause cell and tissue death (Ahuja et al., 2010). Besides this, cold stress has several negative influences like membrane damage, cellular dehydration, inhibition of enzymatic reaction, changes in structures of proteins and macromolecular interactions (Yadav et al., 2010). Plants under the influence of heavy metal stress are also subjected to several physiological and biochemical changes. Prolonged heavy metal exposure to plants affects respiration, photosynthesis efficiency, nutrients uptake and causes membrane damage (Zhang et al., 2009). It was found that abiotic stresses significantly trigger biological, physiological, and molecular changes in plants. Moreover, drought, salt, and temperature stresses can initiate osmotic stresses which retards plant growth. Biotic stress includes different pathogens belonging to different groups like bacteria, fungi, nematodes, insects, and viruses. Uncontrolled biotic stress associated with crop field could cause 100% yield losses. Biotic stress causes several physiological and physical damages like root rot stem rot, plant wilt, seed damages and also affects flowering.

### **3.3 PLANT RESPONSE TO EXTERNAL STRESSES**

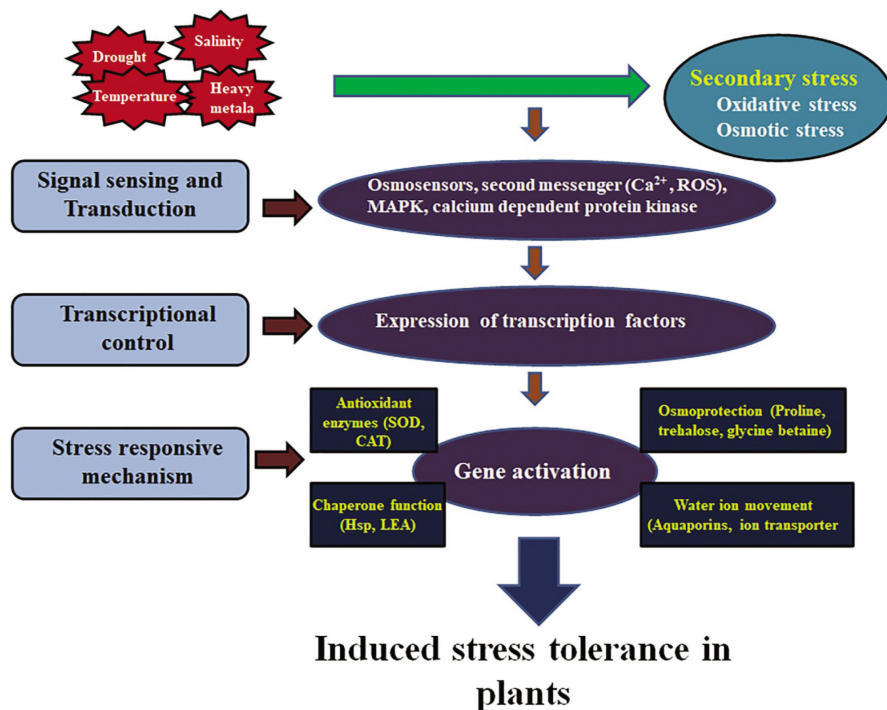
During the stress condition plant suffer from different difficulties and abnormalities in their body which halts growth. Abiotic and biotic stress is the main challenge in the modern sustainable agriculture which needs to be controlled to ensure food security in the near future. With due course of evolution, plants have adopted different protective strategies to minimize the negative

impact and maintain their growth under such condition. It was found that in most of the stress condition overproduction of ROS like hydrogen peroxide ( $H_2O_2$ ), hydroxyl radicals and superoxides occur which has a number of detrimental effects in plants (Nadeem et al., 2013). To minimize the effects of ROS plant developed a complex network of antioxidant enzymes system. Antioxidant enzymes like super oxide dismutase (SOD), catalase, ascorbate peroxidase (APX) and glutathione reductase (GR) improved plant tolerance against environmental stresses. It was reported that plants under stress condition contains much more antioxidant enzymes than plants that are susceptible to stress (Gill & Tuteja, 2010). The action of antioxidant enzymes increases with the increase of stress tolerance in a plant species. Not only the antioxidant enzymes, during the stress period plant also accumulated excess amount of antioxidant metabolites like tocopherols, carotenoids, flavonoids, phenols, etc. (Apel & Hirt, 2004). Increase concentration of salt in the soil creates barriers to nutrients uptake and ion toxicity. To mitigate salt stress plant produces different compatible solutes like trehalose, proline, glycine betaine and many other which enhance tolerance in plant under saline environment (Ashraf et al., 2013). Accumulation of these compatible solutes helps in osmotic adjustment in the plant body that protects water loss and dilutes the concentration of toxic ions (Verma et al., 2021). Plants under temperature stress expressed several stress responsive genes and initiate a complex network of signaling cascade resulting in leaf rolling, alteration of leaf orientation, early maturation and changing membrane structure by altering lipid composition (Hasanuzzaman et al., 2013). Beside biotic factors, biotic stress also causes several damages to plants. Plant protects them from pathogenic attack by producing different hydrolytic enzymes and phytoalexins. Hypersensitive response is another strategy plant adopted during disease progression (Gonzalez-Teuber et al., 2010).

Plant enables different signaling molecules like phytohormones and protein kinases which helps in receiving the stress stimuli and protects plants from different stresses. Beside phytohormones, nitric oxides (NO),  $Ca^{2+}$  and inositol phosphates also help in initiating signaling cascade under stress environment (Hilleary & Gilroy, 2018). These signaling molecules along with the stress stimuli enhance production of different stress-related phytohormones including jasmonic acids (JA), abscisic acid (ABA), salicylic acid (SA) and ethylene. In recent studies, it was reported that NO and  $Ca^{2+}$ -mediated signaling has a crucial role in stress tolerance of plants. ABA-dependent stomata closure and drought stress response is mediated through NO and  $Ca^{2+}$ -dependent signaling pathway (Gulyani et al., 2018).



Mitogen-activated protein kinase (MAPK) helps in the process of diverse type of cellular responses like cell growth and proliferation. Different studies have reported that MAPK has enhanced activity during both biotic and abiotic stress condition (Nanda et al., 2016). An overall response of plants under different stress condition presented in Figure 3.2.



**FIGURE 3.2** Different responses of plant under environmental stress condition.

### 3.4 ENDOPHYTES COLONIZATION AND PLANT GROWTH

Symbiotic relationship is a type of mutualistic interaction between two organisms through which both the organism benefit from each other. Plant and endophytes interaction is a type of symbiotic relationship in which endophytic microorganisms promotes growth and biomass yield in plant and in return endophytes derives nutrients from the host plant for their survival. The term ‘endophytes’ comes from two Greek words ‘endon’ and ‘phyton’ which means within and plants, respectively (Lata et al., 2018). Endophytic organisms mutually colonize inside the plants without forming any symptoms

of infection. Fungal and bacterial endophytes are most unexplored diverse groups of microorganisms that promote both plant growth and stress tolerance (Shiomi et al., 2006). An efficient plant-endophytes interaction needs a successful colonization. Endophytes enter the plant in the same way as pathogens enter the plants, but the main difference is plants develop defense responses and secrete protective molecules against the former. Endophytic organisms may follow some of the important stages for successful colonization inside the plant tissue including host finding, recognition, colonization, and entry into the internal tissue (Rodriguez et al., 2009). Fungal endophytes are generally found in root, stem, and leaf while bacterial endophytes are mainly found in the rhizospheric area. Fungal endophytes belong to two groups, clavicipitaceous endophytes (C-endophytes) and non-clavicipitaceous endophytes (NC-endophytes). C-endophytes are mainly found in grasses and NC-endophytes grow in non-vascular plants, gymnosperms, and angiosperms (Mei & Flinn, 2010). Both bacterial and fungal endophytes take entry through the wound site and natural opening like stomata, lenticels, and hydathodes. Abiotic damages and wounds caused by pathogens are the most promising way for endophytes to take entry. In *Phaseolus vulgaris*, two bacterial endophytes *Enterobacter asburiae* and *Pseudomonas fluorescens* took entry through the damaged tissue caused by pathogen *Rhizoctonia* sp. (Mahaffee & Kloepper, 1997). Endophytic microbes also secrete cell wall degrading enzymes like cellulases, pectinases, proteinases, and hemicellulases. In a study it was found that *Acremonium typhinum* an endophyte secretes a proteinase during colonization in grass *Poa annua* (Lindstrom & Belanger, 1994). Temperature is another important factor in plant-endophyte colonization. Plants with endophytes infection can tolerate 65°C while non-symbiotic association is susceptible to high temperature (Rodriguez et al., 2008). Besides these endophytes can also transmit via seeds and other vegetative organs of plants.

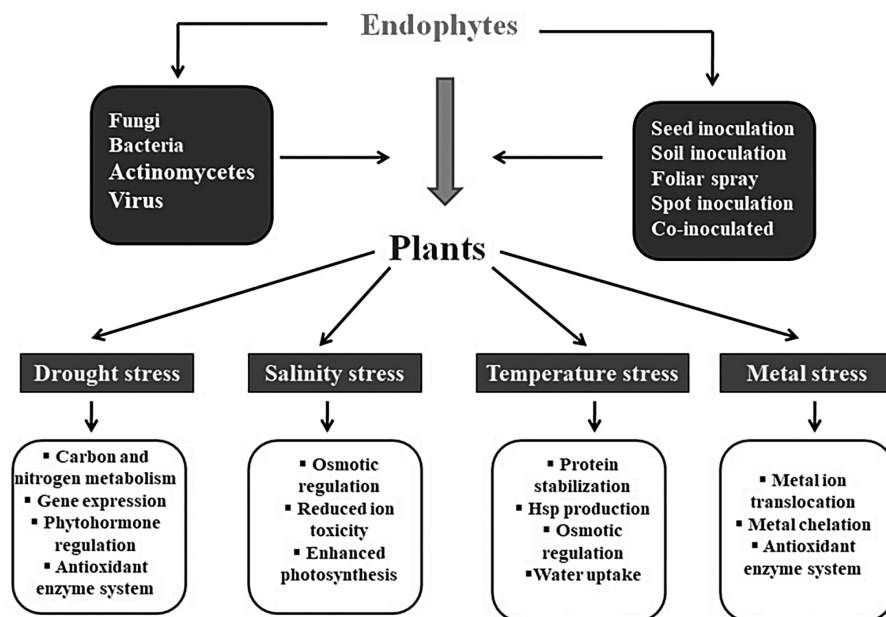
Through plant-endophytes interaction both the organisms draw benefits. Endophytes regulate different physiological processes in the plant which promotes growth and stress tolerance. For instance, bacterial endophytes enhance nitrogen fixation in both leguminous and non-leguminous plants like wheat, rice, and maize (Saravanan et al., 2008). *Gluconacetobacter diazotrophicus* isolated from *Saccharum* sp. promoted growth and nutrient uptake under stress condition (Suman et al., 2005). *Burkholderia kukruiensis* the bacterial endophytes isolated from rice plant improved root and shoot growth and also promoted high grain yield (Mattos et al., 2008). Besides these endophytes also regulate phytohormonal imbalance thus alter physiological

processes and promote growth. Endophytes those especially colonizes the plant root, induce production of indole acetic acid (IAA) an auxin which have several physiological functions like shoot growth, seed germination, root growth, vascular tissue differentiation and promote photosynthesis (Ahmad & Kibert, 2013). In *Solanum nigrum* bacterial endophytes improved growth by the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme and increased production of IAA. IAA also induces plant-endophytes interaction thus promoting better plant growth (Suzuki et al., 2003). During stress condition endophytes limits the production of ethylene, ABA, JA, and gibberellic acid (GA) and promote growth. Endophytic fungi *Phoma herbarum* isolated from salt stressed root of *Glycine* sp. showed improved growth by production of GA. Plants that have poor nutrient uptake machinery in the normal condition, endophytes interaction improved nutrients uptake and promote growth in these plants. Thus, endophyte promotes plant growth and development through three major steps, production of secondary metabolites and phytohormones, improved nutrient acquisition and providing tolerance against biotic and abiotic stresses.

#### **3.4.1 ENDOPHYTES-MEDIATED ABIOTIC STRESS TOLERANCE IN PLANTS**

Endophyte is an essential element of plant symbiosis which helps to mitigate several abiotic stresses in plants. The beneficial roles of endophytes are well studied, and several reports exhibited that under abiotic stress condition these microorganisms alter several biochemical, physiological, and molecular events inside the plant tissue proving better resistance (Nagata et al., 2015; Cherif et al., 2015). For optimal growth in plants, several environmental factors play a key role and alteration of any of these factors can retard growth. Drought, salinity, and temperature are the main abiotic stressors which limits growth. Moreover, these primary stressors can also initiate some secondary stress condition like oxidative and osmotic stresses. Endophytes activate different stress responsive system as soon as the plant receives stress stimuli. Endophyte also induces the expression of different stress responsive genes, biosynthesis of stress related biochemicals including secondary metabolites and phytohormones which minimizes the adverse impact. In pepper plants under stress condition, *Bacillus* sp. and *Arthrobacter* sp. downregulated several genes which promote stress and upregulated several stress defensive genes that protect the plant from harmful effects (Lata et al., 2018). In

cucumber plant two fungal endophytes *Penicillium* sp. and *Phoma glomerata* increased biomass yield by improved uptake of nutrients like magnesium, calcium, and potassium and also reduced sodium ion toxicity under salinity and drought stress (Waqas et al., 2012). An overall mechanism of endophyte-mediated stress tolerance is presented in Figure 3.3.



**FIGURE 3.3** Endophytes-mediated abiotic stress tolerance in plants.

#### 3.4.1.1 ENDOPHYTES AND DROUGHT TOLERANCE IN PLANT

Drought is one of the main stressors responsible for low productivity of many important crops. However, many plants evolved protective mechanisms against drought stress. In this regard, endophytic microorganisms play a significant role in adopting drought. Endophytes colonized rice, tomato, panic grass and dune grass utilized less water under drought stress than plants which do not have endophytic infection. Endophyte helps to accumulate different solutes in the plant tissue and also reduce water conductance and transpiration by altering leaf morphology (Malinowski & Beleskey, 2000). *Rhizobium etli* a bacterial endophyte when inoculated in *Phaseolus vulgaris* induced stress tolerance under drought condition by production of trehalose-6-phosphate synthase enzyme (Suarez et al., 2008). *Azospirillum*

*brasiliense* when inoculated in *Arabidopsis thaliana* improved drought tolerance by upregulating ABA accumulation (Cohen et al., 2015). *Trichoderma* isolated from *Theobroma cacao* induced drought tolerance by altering gene expression pattern (Lata et al., 2018). In maize plant, *Piriformospora indica* minimizes the effects of drought by enhanced proline accumulation, antioxidant enzymes and also induced membrane stability (Xu et al., 2017).

#### 3.4.1.2 ENDOPHYTES AND SALINITY TOLERANCE IN PLANTS

Increasing salinity in the soil is an emerging problem of the modern agricultural system. It was estimated that by the year 2050, 50% of all the arable lands will face severe saline condition (Zhang et al., 2019). In chickpea plant salt tolerance is improved by *Mesorhizobium ciceri* and *Bacillus subtilis* by decreasing oxidative stress and induced production of compatible solute proline (Egamberdieva et al., 2017). In a study it was reported that inoculation of tomato plant with *Pseudomonas fluorescens* and *P. migulae* in a high saline condition induced salt stress tolerance by the activity of ACC deaminase (Ali et al., 2014). Accumulation of excess amount of ROS is a major problem plant generally faces during salinity stress. Endophytes enhance the production of ROS scavengers like catalase, peroxidase, GRs, and superoxide dismutases (SOD). In barley plant application of *P. indica* minimizes the oxidative damages by enhancing antioxidant system in the plant (Baltruschat et al., 2008). *Achromobacter xylosoxidense* promotes growth under saline conditions by decreasing ethylene levels and inducing antioxidant enzymes like SOD and APX in plants (Joe et al., 2012).

#### 3.4.1.3 ENDOPHYTES-MEDIATED TEMPERATURE STRESS TOLERANCE IN PLANTS

Temperature is one of the important factors for plant growth. Uncertain and abnormal temperature exposure damages several crops worldwide. Various studies have supported the fact that endophytes improved plant growth and helped to minimize the impact of chilling and freezing stress (Zhang et al., 2019). In a study when *Lycopersicon esculentum* was inoculated with three bacterial endophyte *Bacillus subtilis*, *B. cereus* and *Serratia* sp. it increases chilling stress tolerance by enhancing antioxidant enzyme system, accumulating soluble sugar and induced expression of stress responsive genes (Wang et al., 2016). In another report it was found that, grass species *Dichanthelium*

*lanuginosum* which contains *Curvularia protuberata* a fungal endophyte, can tolerate up to 65°C soil temperature in Yellowstone National Park (Redman et al., 2002). Fungal endophyte also enhances heat tolerance in *Triticum aestivum* and promotes plant height and grain weight (Lata et al., 2018).

#### **3.4.1.4 ENDOPHYTES-MEDIATED HEAVY METAL STRESS TOLERANCE IN PLANTS**

Nowadays, heavy metal contamination in soil and underground water is a common phenomenon due to expansion of industrialization and urbanization. Application of chemical fertilizer by the farmers is also increase heavy metal contamination in soil. Accumulations of heavy metal in the plant body have several detrimental effects that limit growth. *Exophiala pisciphila* inhabiting in maize root limits cadmium (Cd) stress by activating antioxidant enzymes (Wang et al., 2016). *Pseudomonas* and *Gigaspora* can induce metal stress tolerance by altering the level of ACC deaminase which helps to manipulate the ethylene levels in plants (Friesen et al., 2011). Tobacco plants inoculated with *Acrocalymma vagum* decreased the amount of heavy metals like Cd, lead (Pb) and Arsenic (As) and also limits the translocation of these heavy metals in the plant (Jin et al., 2017).

Plants might face more than one stress condition simultaneously. It was reported that endophytes can improve tolerance in plants against salinity, drought, and temperature stress simultaneously (Zhang et al., 2019). In Table 3.1, we have presented different endophytic microorganisms that contribute to plant growth and development under different stress conditions.

#### **3.4.2 ENDOPHYTES-MEDIATED BIOTIC STRESS MANAGEMENT IN PLANTS**

In plants, biotic stresses are mainly characterized by the attack of various pathogens and insect pests which causes a negative impact on plant growth, leading to major yield loss. Endophytic microorganisms showed interaction with fungal pathogens, nematodes, and many other plant pathogens giving protection to plants (Chadha et al., 2015). In several studies fungal endophytes exhibited significant antimicrobial activity and also inhibit several plant pathogens through antagonism (Rodríguez et al., 2008). Fungal endophytes are used as biocontrol agents for several pathogenic microbes. For instance, *Penicillium citrinum* is used to control the destructive pathogen

**TABLE 3.1** Different Endophytes, Their Host, and Stress Tolerance Mechanisms

Type of Stress	Endophytes	Host Plant	Mechanism	References
Drought	<i>Bacillus subtilis</i>	<i>Brachypodium distachyon</i>	Upregulation of drought responsive genes	Gagne-Bourque et al. (2015)
	<i>Pantoea alhagi</i>	<i>Arabidopsis</i> and wheat	Accumulation of soluble sugars, decreased accumulation of proline and protects chlorophyll contents from degradation	Chen et al. (2017)
	<i>Sinorhizobium meliloti</i>	<i>Medicago sativa</i>	Enhanced antioxidant enzymes system	Naya et al. (2007)
	<i>Trichoderma harzianum</i>	<i>Oryza sativa</i>	Enhanced water uptake by upregulation of aquaporins	Pandey et al. (2016)
	<i>Phoma</i> sp.	<i>Pinus tabulaeformis</i>	Increased production of superoxide dismutase and catalase	Zhou et al. (2021)
Salinity	<i>Piriformospora indica</i>	<i>Brassica campestris</i>	Increased production of antioxidant enzymes	Sun et al. (2010)
	<i>Epichloë bromicola</i>	<i>Hordeum brevisubulatum</i>	Increased glycine betaine content, antioxidant content and photosynthesis	Chen et al. (2018)
	<i>Pantoea agglomerans</i>	<i>Zea mays</i>	Improved water uptake by inducing membrane ions channel	Gond et al. (2015)
	<i>Streptomyces</i> sp.	<i>Oryza sativa</i>	Decreased ethylene level and increased accumulation of proline and Ca <sup>2+</sup> ions	Jaemsaeng et al. (2018)
	<i>Curvularia</i> sp.	Poplar plant	Induced antioxidant enzymes like SOD and APX	Pan et al. (2018)
Temperature	<i>Piriformospora indica</i>	<i>Hordeum Vulgare</i>	Increased antioxidant content and reduced ion toxicity	Baltruschat et al. (2008)
	<i>Burkholderia phytofirmans</i>	<i>Arabidopsis thaliana</i>	Induced cold response signaling and photosynthetic pigments	Su et al. (2015)
	<i>Pseudomonas vancouverensis</i>	<i>Solanum Lycopersicum</i>	Membrane stabilization and induced proteins that protects chilling stress	Subramanian et al. (2015)

**TABLE 3.1** (Continued)

Type of Stress	Endophytes	Host Plant	Mechanism	References
Heavy metal (As)	<i>Staphylococcus arlettae</i>	<i>Brassica juncea</i>	Enhanced arsenic reductase activity, increased phosphorus in soil	Srivastava et al. (2013)
Heavy metal (Cd)	<i>Neotyphodium uncinatum</i>	Rye grass	Reduced leaf expansion	Ren et al. (2006)
Heavy metal (Cd, Mn)	<i>Bacillus</i> sp.	<i>Sorghum bicolor</i>	Accumulation of root tillers and biomass	Lata et al. (2018)
Heavy metal (Cd, Pb, Zn, As)	<i>Pseudomonas koreensis</i>	<i>Miscanthus sinensis</i>	Induced antioxidant enzymes and limited translocation of Pb, Cd, and Zn	Babu et al. (2015)
Osmotic	<i>Pseudomonas</i> sp.	<i>Nicotiana tabaccum</i>	Induced genes that helps to degrade starch	Sarma et al. (2011)



*Fusarium oxysporum*, through the production of specific enzymes (Ting et al., 2012). During the pathogenic attack fungal endophytes helps plant to produce phytoalexins, secondary metabolites and phytohormones providing defensive response in plants (Dastogeer et al., 2020). Beside fungal endophytes, bacterial microorganism also helped plants by providing protection against several pathogenic attacks. *Pseudomonas putida* and *Serratia plymuthica* can inhibit two fungal pathogen *Rhizoctonia solani* and *Verticillium dahliae* by secreting antifungal biochemicals (Zhang et al., 2019).

### 3.5 PGPR AND PLANT GROWTH

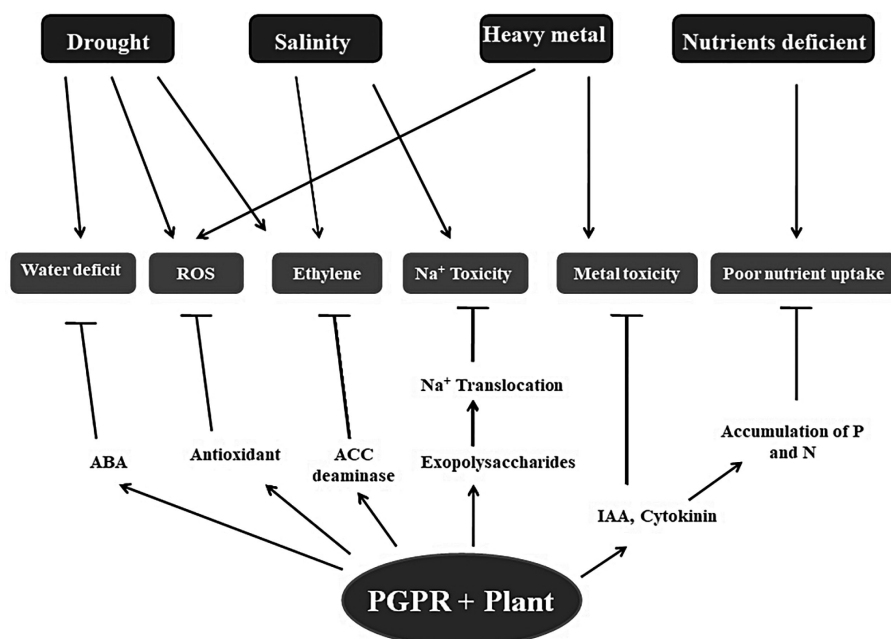
Rhizosphere is the area in the soil that is unique in nature as it supports the highest microbial diversity and activity. Rhizosphere is greatly influenced by the activity of plant roots. A great proportion of microorganisms occupies the root rhizosphere area that promotes plant growth are known as plant growth promoting rhizobacteria (PGPR). Roots secrete several organic solutes, sugars, and chemicals which are needed for the survival of PGPR and in return PGPR promotes plant growth by regulating different biochemical, physiological, and anatomical processes (Nadeem et al., 2013). In addition to plant growth, these beneficial bacteria also provide tolerance against adverse environmental conditions like drought, salinity, and heavy metals stress. PGPR-mediated plant growth includes both direct and indirect methods. Direct mechanism involves the production of beneficial compounds by the bacterium that helps in nutrients uptake and fixing biological nitrogen. An indirect mechanism occurs when PGPR inhibits pathogenic attacks in the plant. They reduce the impact of pathogen attack by producing substances that induce resistance in plants and also produce siderophores which limits iron availability for the pathogens. Beside these two mechanisms, PGPR has a striking ability to produce different enzymes including ACC deaminase and chitinase. During the plant growth PGPR enhances production of IAA, cytokinin, ABA, siderophores, hydrogen cyanide and rhizobitoxine which not only induce plant growth but also provide resistance against biotic and abiotic stresses (Nadeem et al., 2013; Kumar et al., 2019). Rhizobitoxine induce nodule formation by inhibiting the level of ethylene which has a negative impact on nodulation. In addition to this ACC deaminase is a very important enzyme for plant growth under different stress conditions like drought, salinity, and heavy metal stress. Bacteria those produce ACC deaminase promote plant growth under abiotic stress condition by cleaving

the precursor of ethylene into ammonia and  $\alpha$ -ketobutyrate resulting low level of ethylene in plant. IAA is the type of auxin class of phytohormone which has a key role in plant growth. PGPR those produces IAA promotes root and shoot growth and increased water uptake under saline condition (Kumar et al., 2019). During stress condition ABA plays an important role in stomata closure that protects plants from losing water. Production of cytokinin is another common feature of PGPR. Cytokinin positively regulates cell growth, root and shoot development and tissue differentiation. PGPR, producing a high concentration of cytokinin, potentially improves tolerance in plants against stress conditions. For instance, *Bacillus subtilis* enhanced drought resistance and shoot biomass in lettuce plant by elevating the level of cytokinin (Arkhipova et al., 2007). Several genera of PGPR are also used in the agricultural sector as bio-fertilizer which facilitate enhanced nutrient uptake. *Oscimium basilicum* inoculated with *Cronobacter dublinensis*, *Bacillus* sp. and *Pseudomonas monteilii* improved nutrient uptake under stress condition (Rakshapal et al., 2013).

### **3.5.1 PGPR-MEDIATED ABIOTIC STRESS TOLERANCE IN PLANTS**

Application of PGPR to manage abiotic stress in plant is now a widely followed strategy. The first evidence of PGPR mediated stress tolerance was found in *Arabidopsis thaliana* when *Paenibacillus polymyxa* improved drought tolerance in the plant (Timmusk & Wagner, 1999). After that, various studies have well established the fact that PGPR have several beneficial effects on plants under abiotic stress condition. The main mechanisms through which they promote stress tolerance are producing growth hormones, inhibiting the effects of ethylene, enhanced nutrients uptake, facilitate nitrogen fixation, producing antimicrobial molecules and minimizes effects of ROS (Ndeem et al., 2013). During stress condition ethylene level needs to be maintained normal to achieve normal growth. PGPR maintains ethylene level by producing ACC deaminase enzyme. PGPR binds to the root surface and produce IAA which helps to elevate the concentration of ACC deaminase in the root vicinity and promote root growth under drought situation (Glick et al., 1998). Under saline environment plants cope with nutritional imbalance and ion toxicity. It is very essential to maintain a high ratio of  $K^+/Na^+$  in the plant to overcome salinity stress. Exopolysaccharides produced by certain strains of PGPR limits the accumulation of excess  $Na^+$  ions, thus maintaining a high  $K^+/Na^+$  ratio inside the cell (Glick et al., 2007). PGPR improved plants

growth under heavy metal stress by producing antioxidant enzymes, IAA, and ACC deaminase (Kumar et al., 2019). The overall mechanisms of abiotic stress tolerance in plants by PGPR are summarized in Figure 3.4.



**FIGURE 3.4** PGPR-mediated stress tolerance in plants.

### 3.5.1.1 PGPR-MEDIATED DROUGHT TOLERANCE IN PLANT

Among different abiotic stresses, drought stress have several negative impacts on plants, and it is one of the main factors that only inhibit growth but also responsible for oxidative damages. Though modern biotechnological techniques improved plant resistance to drought by producing transgenic crops but still growth promoting rhizobacteria plays a pivotal role during the stress period. In drought scenario, the main problem in the plant body is the production of ROS and ethylene which limits root growth leading to impaired nutrients uptake. As PGPR has the ability to produce ACC deaminase and antioxidant enzymes thus it can inhibit the negative impact and permits continue growth (Kumar et al., 2019). *Pseudomonas fluorescens* when inoculated in *Catharranthus roseus* it improved drought resistance and also increased secondary metabolites contents (Nadeem et al., 2013). Similarly, in pea plants PGPR induced drought tolerance through the activity of

ACC deaminase enzyme (Glick et al., 2007). Inoculation of *Enterobacter* sp. with endophytes improved drought resistance in maize, wheat, sugarcane, and sunflower plant by enhanced photosynthetic efficiency and water uptake (Kasim et al., 2013).

### 3.5.1.2 PGPR-MEDIATED SALT TOLERANCE IN PLANT

Increasing amount of salinity in the soil atmosphere greatly reduce crop productivity. Salinity is currently one of the major challenges in the sustainable agriculture system. Almost all the major crops grown part of the earth is facing more or less saline condition affecting plant growth. Production of salt tolerant crops can be achieved by genetic engineering but till date very few salts tolerant crops has been introduced. Thus, PGPR is used as an alternative strategy to induce salt tolerance in plants. Microorganism living in the root vicinity promotes growth by regulating source-sink interaction, biochemical metabolism, leaf senescence and biomass production. PGPR regulate signaling cascade in the root and shoot through which enhanced the production of stress responsive phytohormones under salinity stress (Ghanem et al., 2011). Under extreme saline condition plant faces osmotic inhibition of water uptake. Accumulation of excess  $\text{Na}^+$  ions under saline environment decrease the amount of  $\text{Ca}^{2+}$  and  $\text{K}^+$  ions which creates ionic toxicity. In a study, *Azospirillum brasilense* improved shoot elongation in wheat seedlings under saline environment (Creus et al., 1997). Under saline condition, PGPR inhibits ROS and ethylene production via antioxidant enzymes and ACC deaminase enzymes.

### 3.5.1.3 PGPR-MEDIATED HEAVY METAL TOLERANCE IN PLANT

Heavy metal contamination in the soil affects plant growth by developing impaired metabolism system. Cd, Zn, Pb, and Ni are some of the principal heavy metals that are released by different industrial waste materials contributing to the heavy metal toxicity in the soil. PGPR has several key functions which help to detoxify the impact of heavy metals in plants. PGPR improves water uptake and mineral solubilization in the plant under heavy metal contamination promoting plant growth (Majeed et al., 2015). PGPR promotes IAA and cytokinin production which helps plant to expand their roots in the soil to uptake minerals. Moreover, rhizosphere bacteria secrete some organic acids which increase the amount of solubilized phosphorus.

Metal chelation is another attribute of PGPR under extreme heavy metal contamination (Glick et al., 2007). Under Zn contamination plants inoculated with *Pseudomonas aeruginosa* showed higher gene expression for antioxidant enzymes like catalase and SOD than those un-inoculated plants (Noctor & Foyer, 1998). It was also reported that some PGPR strains can accumulate metals thus reducing their availability in the soil rhizosphere (Naddem et al., 2013). In Table 3.2, some of PGPRs and their effects on plant stress tolerance are listed.

### **3.5.2 PGPR-MEDIATED BIOTIC STRESS MANAGEMENT IN PLANTS**

Inhibition of different plant pathogens by PGPR enabled their use as a potential biocontrol agent. PGPR increases host resistance by several mechanisms, including secreting different cell wall degrading lytic enzymes, improved nutrients availability, and inducing systemic resistance (Bhattacharyya & Jha, 2012). In some studies, the biocontrol activity of PGPRs is well established in the plant tomato and pepper (Mayak et al., 2004). *Pseudomonas polymyxa* can inhibit fungal pathogens like *Fusarium* sp. and *Botrytis* sp. which are responsible for several destructive diseases in many crops (Kumar et al., 2019). In another report it was found that, *Bacillus amyloliquefaciens* can inhibit wheat pathogen *Fusarium gaminearum* by reducing the production of deoxynivalenol (DON) a mycotoxin produced by the fungus (Shi et al., 2014). This PGPR was also found to be effective in eggplant against *Fusarium* sp. (Chakraborty et al., 2021). Another aspect of is the inhibition of pathogens by exhibiting antagonistic mechanism. Rhizobacteria can produce many toxic and protective secondary metabolites which help to inhibit disease progression. Though this mechanism is not clearly understood but it was suggested that most of the protective secondary metabolites are non-ribosomal peptides and polyketides (Mongkolthanaruk, 2012). For instance, *Bacillus* and *Paenibacillus* exhibited antagonistic mechanism by producing secondary metabolites in many crop species (Cawoy et al., 2013).

## **3.6 ARBUSCULAR MYCORRHIZAL FUNGI (AMF) AND PLANT GROWTH**

Arbuscular mycorrhizal fungi (AMF) are the microorganism present in the soil showing obligate symbiotic relationship with plants. AMF are the most pervasive microorganisms that are found in almost 90% of plants and crop

**TABLE 3.2** PGPR and Their Effects on Plants Abiotic Stress Tolerance

Type of Stress	PGPRs	Host	Response in Plants	References
Drought	<i>Burkholderia phytofirmans</i>	Maize	Increased photosynthetic activity and improved water uptake.	Naveed et al. (2014)
	<i>Achromobacter piechaudii</i>	<i>Solanum lycopersicum</i> and <i>Capsicum annuum</i>	Induced plant growth under low water availability by the action of ACC deaminase which lowers ethylene level in root.	Mayak et al. (2004)
	<i>Azospirillum</i> spp.	Wheat	Provide plants growth by inducing IAA and cytokinin.	Kumar et al. (2019)
Salinity	<i>Bacillus</i> spp.	Lettuce	Increased photosynthetic activity.	Vivas et al. (2003)
	<i>Pseudomonas fluorescens</i>	<i>Arachis hypogaea</i>	Improved salt tolerance and reduced Na <sup>+</sup> toxicity.	Nadeem et al. (2013)
	<i>Azospirillum</i>	Wheat	Improved plant height, grain yield and photosynthetic pigments.	Nia et al. (2012)
	<i>Pseudomonas</i> spp.	Rice	Germination of seedlings.	Kumar et al. (2019)
	<i>Azotobacter</i>	Maize	Induced uptake of P and K, lowered Na <sup>+</sup> intake.	Rojas-Tapias et al. (2012)
	<i>Pseudomonas aeruginosa</i>	Sunflower	Enhanced plant growth.	Kumar et al. (2019)
	<i>Xanthobacter autotrophicus</i>	Eggplant	Improved minerals uptake.	Abd El-Azeem et al. (2012)
	<i>Klebsiella oxytoca</i>	Cotton	Maintained plant growth by producing malondialdehyde, proline, and chlorophyll-a.	Wu et al. (2014)
	<i>Piriformospora indica</i>	Wheat	Enhanced salt tolerance by producing photosynthetic pigments and soluble sugars (organic solutes)	Zarea et al. (2012)

**TABLE 3.2** (Continued)

Type of Stress	PGPRs	Host	Response in Plants	References
Heavy metal	<i>Rhodococcus</i> spp.	<i>Brassica juncea</i>	Reduced Cd toxicity in plants by reducing Cd concentration in plant.	Nadeem et al. (2013)
	<i>Rhizobium trifolii</i> and <i>Brevibacillus brevis</i>	Clover	Induced growth by accumulating N and P under nickel toxicity.	Vivas et al. (2003)
	<i>Pseudomonas brassicacearum</i>	<i>Pisum sativum</i>	Induced root growth under Cd contaminated soil.	Safronova et al. (2006)
	<i>Pseudomonas aeruginosa</i>	Wheat	Enhanced plant growth under Zn contaminated soil by increasing leaf chlorophyll and soluble proteins.	Nadeem et al. (2013)

plant (Diagne et al., 2020). AMF fungi is an important constituent of the soil contributing 5–36% total biomass of the soil and 9–55% total microorganisms that are living in the soil (Olson et al., 1999). In plant, there are mainly two types of mycorrhizal association are found namely *Arbuscular mycorrhizae* (AM) and ectomycorrhizae, among which AM is the most abundant fungi found in the soil biomass. AM fungi belong to the subkingdom Mucoromyceta and phylum Glomeromycota which contains 11 families, 25 genera and about 250 species (Spatafora et al., 2016). AMF forms a hyphal network in the root cells of the plant known as arbuscle which helps them to draw foods from the host. These hyphal structures also penetrate the soil and enables plant to uptake minerals and nutrients providing growth (Nadeem et al., 2013). Aggregation of the mycelial structure increases both the root surface and root length that helps plant to reach more nutrients pools which are unavailable for the uncolonized root system. Moreover, sometimes AMF mycelia are function as nutrients absorbing organs in addition to root. Extension of the hyphal network also facilitates soil aggregation thus improves the physical properties of the soil. AMF also contribute to the soil fertility by increasing the availability of different macro and micro-nutrients like nitrogen, phosphorus, copper, and magnesium. It has been reported that almost 80% phosphorus uptake is facilitated by the AM fungi in plants (Allen, 2011). It was found that in legumes plant AM fungi in addition with *Rhizobium* sp. helped in better nutrients uptake and also increased the efficacy of *Rhizobium* sp. to support plant under stress condition. The fungal symbionts also positively regulate nodule formation in legumes (Guo & Haung, 2010). Furthermore, these AM fungi can store several nutrients, thereby giving protection to plants under nutrient deficit condition. AMF fungi could promote nutrient cycling and increases nutrients in the soil by creating soil environment favorable for other beneficial microorganisms or by synergizing with other rhizobacteria and endophytes (Nadeem et al., 2013). AM fungi produce a glycoprotein glomalin which is secreted from the hyphae. Glomalin binds nitrogen and carbon with the soil particles, thereby increasing soil carbon content and nutrients availability. Plant water relation is directly associated with glomalin content present in soil, contributing to approximately 20% total water uptake by plants (Gadkar & Rilling, 2006). AMF is also used as broad spectrum biofertilizer in different agricultural land providing high yields, better nutrients uptake, improved water status and enhanced soil quality (Diagne et al., 2020).

In *Cucurbita maxima* inoculation with AM fungi significantly improved metabolism including carbohydrate, crude protein and fiber content in the root

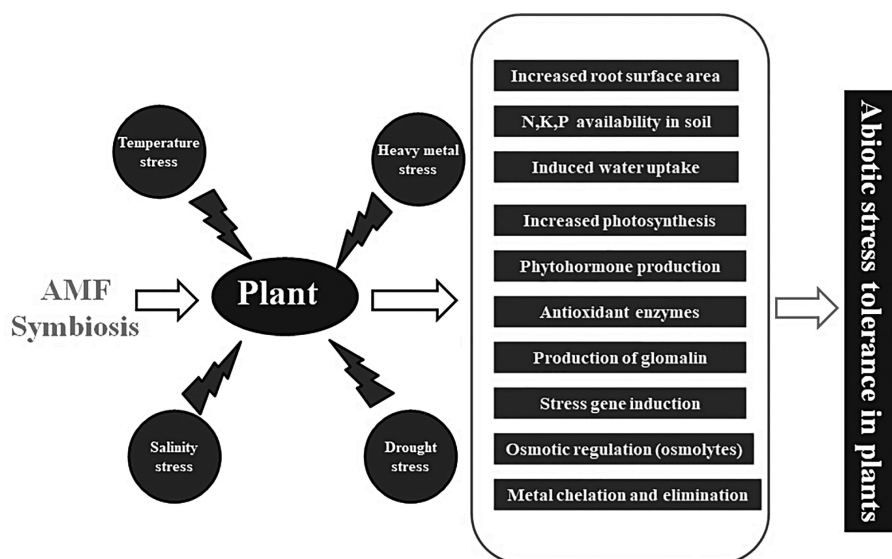


and shoot tissue thereby improving plant growth (Al-Hmoud & Al-Momany, 2017). Mycorrhizal fungi colonize in the watermelon plant improved water uptake, nutrient availability, and fruit quality (Keya et al., 2003). In another study it was found that, three AMF fungi *Acaulospora longula*, *Scutellospora heterogama* and *Funneliformis mosseae* exhibited positive effects on growth of carrot plant (Kim et al., 2017). Symbiotic fungal colonization in the root of barley and bread wheat improved Zn deficiency (Coccina et al., 2019). In sorghum plant enhancement of biomass and nitrogen, phosphorus, and potassium uptake is greatly influenced by the colonized mycorrhiza. It was also suggested that in this plant nutrients uptake is not only depends on the fungal partner but also depends on different nutrient uptake pathway selected by the host plant (Nakmee et al., 2016). In laboratory condition micro-propagated *Prunus cerasifera* inoculated with *Rhizoglosum intraradices* and *Funneliformis mosseae* exhibited better growth and 100% survival than those which are uninoculated with these fungal symbionts (Diagne et al., 2020). In addition to AMF mediated plant growth, these symbiotic mycorrhizal fungi can also interact with several other microorganisms residing soil atmosphere and exhibit plant growth regulating functions. In general, AMF promotes plant growth by not only enhancing nutrient uptake but also minimizes the negative impacts of several biotic and abiotic stress conditions.

### **3.6.1 AMF-MEDIATED ABIOTIC STRESS TOLERANCE IN PLANTS**

Having a number of positive effects on plants growth and development AMF is also well known for their role in managing plant stress tolerance against different abiotic factors. Various reports have already shown that, AMF has induced plant growth and tolerance under several adverse environmental conditions like drought, salinity, flooding, temperature, and heavy metal stresses (Allen et al., 2011; Nadeem et al., 2013; Diagne et al., 2020). In the presence of stress stimuli AMF fungi living in the plant root change their composition. These changes result in decreased diversity of the fungi and form an AMF community having similar type of species that are tolerant to that specific stress condition. Change in AMF community helps plant to respond more vigorously against the adverse condition (Bennett & Classen, 2020). With changing of climate this response by the AMF become stronger proving better resistance in plants. AMF adaptation under stress condition is not dependent on the host plant. Thus, colonization of AMF improves plants stability under drought,

salinity, and temperature stress (Nakmee et al., 2016). AMF mediated abiotic stress tolerance involved a diverse type of mechanisms including: (i) facilitation of nutrients and water uptake; (ii) accumulation of organic solutes, stress hormones and expression of stress responsive genes; (iii) alteration in plant physiology like osmotic adjustment, photosynthesis, gaseous exchange; (iv) improved antioxidant enzyme system; and (v). heavy metal chelation and elimination of pollutants from soil (Diagne et al., 2020). An overall mechanism of AMF mediated abiotic stress tolerance is summarized in Figure 3.5.



**FIGURE 3.5** Possible mechanism of AMF mediated abiotic stress tolerance in plants.

### 3.6.1.1 AMF AND DROUGHT STRESS TOLERANCE

Drought stress is one of the major factors responsible for low productivity of crop plants. Due to the low water availability in the soil, plant closes their stomata which eventually decreases photosynthetic rate as gaseous exchange get hampered. During drought AMF improved plant fitness through both drought mitigation and drought tolerance. AMF enhanced water uptake ability in plants which helps in drought mitigation and a combination of direct benefits provide strength to plants for drought tolerance (Bernardo et al., 2019). Root colonization of AMF increased root surface area for water absorption and also helps to penetrate a greater extent of the soil. Several

studies have shown that root colonization of AMF improved plant growth under drought condition by enhancing nutrients and water uptake ability. For instance, root colonization of AM fungi in *Allium cepa*, *Trifolium repens*, *Lactuca sativa* and *Triticum aestivum* improved growth under drought condition by expanding root surface area for water uptake (Diagne et al., 2020). Under drought condition, AM fungi induced leaf water potential, stomatal conductance, improved gaseous exchange, transpiration, and photosynthesis in plants. AMF minimizes the harmful effects of drought stress on photosystem II reaction center (Zhu et al., 2012). AMF also improved water uptake by producing phytohormones and organic solutes. During stress, ABA plays an important role in plant. ABA modulates several hormonal signaling like root water conductivity, stomatal opening, and expression of water channel across the membrane. Colonization of AMF lowers the ABA level in the root which helps in water retention (Yooyongwech et al., 2013). Beside ABA, AMF also induced auxin and strigolactones that improved water stress response in plants. Another aspect of AMF is an accumulation of organic solutes like proline, glycine betaine and sugars which helps to adjust the osmotic potential of plant under drought condition. Antioxidant enzymes produced by the AMF reduced ROS accumulation protects plants from oxidative damages under drought stress (Laxa et al., 2019). Beside drought tolerance, AMF also induced flooding tolerance in plants. It was observed that in wet soil high diversity of AMF are formed. Flooding tolerance in plant is directly associated with high nutrient uptake and good photosynthetic efficiency (Bao et al., 2019).

### 3.6.1.2 AMF AND SALINITY TOLERANCE

AMF can adopt saline condition efficiently. AMF colonization improved several plants growth under extreme saline conditions (Table 3.3). It has been well established that AM fungi can modulate several biochemical, physical, and physiological processes in plants. We already know that AMF can improve water uptake and increase nutrients availability in soil. This attribute of AM fungi induced salt tolerance in many plants including *Zea mays*, *Triticum aestivum* and tomato (Estrada et al., 2013). In addition to this, AM inoculation in plant root under saline condition accumulates osmoregulators like proline and sugars, improved water conductance of the root tissue, enhanced photosynthesis activity and reduced  $\text{Na}^+$  ion toxicity by maintaining the balance of  $\text{K}^+/\text{Na}^+$  ratio (Li et al., 2020). Tomato plant inoculated with *Funneliformis mosseae* under saline condition improved

Fe, P, K, and Cu content of shoot, increased plant biomass and fruit yields (Al-Karaki et al., 2006). *Zea mays* inoculated with three strains of fungal symbionts improved plant biomass under salt stress by enhancing proline and potassium content in the shoot (Estrada et al., 2013).

### 3.6.1.3 AMF AND TEMPERATURE TOLERANCE

An extreme temperature (cold and heat) is another negative regulator of plant growth. AM fungi colonization can induce temperature tolerance (Table 3.3) in plants by accumulating osmolytes, enhancing photosynthesis, improving nutrients and water uptake, and minimizing ROS accumulation (Nadeem et al., 2013). At high temperature AMF helps plants by extension of root surface area producing a mycelial network that ensures water uptake and protects photosynthetic apparatus from damage (Zhu et al., 2012). During the period of temperature stress, AMF reduced the emission of N<sub>2</sub>O from plant by increasing nitrogen in the soil. It was reported that in low temperature plants those are inoculated with AMF exhibited better growth and biomass accumulation than those un-inoculated plants. For evidence, when *Hordeum vulgare* inoculated with AMF under low temperature stress showed better yield and biomass production (Hajiboland et al., 2019). Therefore, AMF can induce tolerance in plants under extreme environmental conditions.

### 3.6.1.4 AMF AND HEAVY METAL TOLERANCE

Mining and industrial areas are more prone to heavy metal contamination. Plants those are adopted these environments exhibited a greater proportion of AMF colonization in the root. AMF are used as a heavy metal stress reducing agents in contaminated soil area as it helps to eliminate metal from the soil thus reducing heavy metal toxicity in plants (Wang, 2017). AMF provides heavy metal remediation as their hyphae has the metal binding ability that reduces the bioavailability of metals like Cd, Co, Zn, and Pb (Audet & Charset, 2007). When maize plant is inoculated with *Glomus* sp. under heavy metal contaminated soil it showed better biomass yields and higher P, K, and Mg content in the root (Kaldorf et al., 1999). In a recent study it was found that AMF can induce expression of some metal transporter. For instance, in *Rhizophagus irregularis* a Zn transporter GintZn T1 was reported (Gonzalez-Guerrero et al., 2016). Different AM fungi and their role in abiotic stress tolerance in different plant species are presented in Table 3.3.

**TABLE 3.3** Different AMF and Their Role in Abiotic Stress Tolerance in Plants

Type of Stress	AM Fungal Strains	Host	Stress Response	References
Drought	<i>Glomus intraradices</i>	<i>Solanum lycopersicum</i>	Improved N and P uptake, increased fruit biomass.	Subramanian et al. (2006)
	<i>Glomus clarum</i>	<i>Lycopersicon esculentum</i>	Increased leaf area, root, and stomatal conductivity, improved photosynthetic efficiency.	Dell'Amico et al. (2002)
	<i>Glomus intraradices</i>	<i>Sorghum bicolor</i>	Enhanced grain yields.	Alizadeh et al. (2011)
	<i>Rhizophagus irregularis</i>	<i>Zea mays</i>	Improved water translocation in plant.	Barzana et al. (2012)
	<i>Rhizophagus fasciculatus</i>	<i>Triticum aestivum</i>	Improved osmotic adjustment and leaf water conductance.	Allen et al. (2011)
	<i>Rhizophagus irregularis</i>	<i>Trifolium repens</i>	Increased proline content, nutrients like P and N and improved dry weight and water uptake.	Ortiz et al. (2015)
	<i>Glomus etunicatum</i>	<i>Pistacia vera</i>	Increased chlorophyll content, nutrient concentration, accumulation of proline, soluble sugar, increased antioxidant enzymes.	Abbaspour et al. (2012)
	<i>Glomus mosseae</i>	<i>Vigna radiata</i>	Increased leaf nutrient contents and improved water relation.	Nadeem et al. (2013)
Flooding	<i>Glomus intraradices</i>	<i>Pterocarpus officinalis</i>	Improved phosphorus content.	Fougnies et al. (2006)
	<i>Glomus geosporum</i>	<i>Aster tripolium</i>	Accumulated proline and soluble sugar and proteins.	Solís-Rodríguez et al. (2020)

**TABLE 3.3** (Continued)

Type of Stress	AM Fungal Strains	Host	Stress Response	References
Salinity	<i>Glomus</i> spp.	Wheat	Increased plant biomass.	Daei et al. (2009)
	<i>Claroideoglomus etunicatum</i>	<i>Glycine max</i>	Improved plant growth by producing auxin.	Hashem et al. (2016)
	<i>Funneliformis mosseae</i>	<i>Gossypium arboreum</i>	Increased nutrient content (P) and plant biomass	Tian et al. (2004)
	<i>Rhizophagus irregularis</i>	<i>Digitaria eriantha</i>	Increased accumulation of antioxidant enzymes, stomatal conductance and reduced H <sub>2</sub> O <sub>2</sub> formation in root	Pedranzani et al. (2015)
	<i>Claroideoglomus etunicatum</i>	<i>Cucumis sativus</i>	Increased nutrient contents (P, K, Ca, Mg, Mn, Zn), antioxidant enzymes, photosynthetic pigments, and reduced Na <sup>+</sup> uptake.	Diagne et al. (2020)
Temperature	<i>Funneliformis mosseae</i>	<i>Elymus nutans</i>	Reduced oxidative damages and enhanced chlorophyll contents.	Chu et al. (2016)
	<i>Funneliformis</i> sp.	<i>Zea mays</i>	Increased photosynthetic efficiency by protecting photosystem II from damage.	Mathur & Jajoo (2020)
	<i>Glomus versiforme</i>	<i>Hordeum vulgare</i>	Increased survival under low temperature stress.	Diagne et al. (2020)
	<i>Ribes fasciculatum</i>	<i>Cyclamen persicum</i>	Increased antioxidant enzyme production and biomass under high temperature.	Maya et al. (2013)
Heavy metal	<i>Glomus versiforme</i>	<i>Lonicera japonica</i>	Decreased Cd concentration in root and shoot.	Jiang et al. (2016)
	<i>Glomus mosseae</i>	<i>Trifolium pratense</i>	Decreased Zn toxicity in root and shoot.	Li & Christie (2001)
	<i>Glomus intraradices</i>	<i>Solanum lycopersicum</i>	Reduced Cd contamination and oxidative damages.	Hashem et al. (2016)
	<i>Funneliformis mosseae</i>	<i>Trigonella foenum-graceum</i>	Enhanced heavy metal translocation and increased mineral uptake.	Kelkar et al. (2013)

### 3.6.2 AMF AND BIOTIC STRESS MANAGEMENT IN PLANTS

Symbiotic noninfectious microorganisms living in the plant root and rhizosphere is known to have a potential role in managing different plant pathogens. The role of AMF in reducing the damage caused by the pathogen is well-studied by different researchers. AMF-colonized plant successfully inhibited several pathogens in field conditions, and in some diseased plant growth remains unaffected. Charcoal root rot disease of *Glycine max* causes by the pathogen *Macrophomina phaseolina* resulting huge economic loss every year, can be controlled by applying AM inoculation (Spagnoletti et al., 2020). In plant mycorrhizal colonization controls disease progression and disease severity by providing a resistance to plant known as mycorrhizal-induced resistance (MIR). MIR is similar to systemic acquired resistance that gives systemic protection from pathogen attacks (Nguvo & Gao, 2019). AMF induced resistance in plant by different mechanisms including changing root morphology and structures, producing antioxidant enzymes, improving nutrients status in the host plant, enhancing photosynthesis, and changing microbial diversity in the mycorrhizosphere (Cameron et al., 2013). Colonization of tomato plant root by *Funneliformis mosseae* improved resistance against two nematode pathogen *Pratylenchus penetrans* and *Meloidogyne incognita*. Besides this, inoculation of *F. mosseae* also inhibits two fungal pathogen *Fusarium oxysporum* and *Alternaria solani* in tomato plant (Vos et al., 2012). Several reports also revealed that besides fungal and nematodes, AMF fungi can control several herbivorous insects and invasive plants like *Striga* (Diagne et al., 2020).

### 3.7 CONCLUSION AND FUTURE PROSPECTS

From the above discussion, it is evident that adverse environmental condition and rapid climate change affects plants growth and development by causing several biochemical, physical, and physiological changes. Not only the abiotic factors, biotic factors like presence of disease are also hampered plant growth. Many soil residing microorganisms like endophytes, rhizobacteria, and mycorrhizal fungi help to minimize the negative impacts of these stresses. Though many researchers are well described the role of these microorganism individually but there is also presence of evidence that application of combination of these microbes can improve stress tolerance in plant even better. Another important aspect of the usefulness of these microbes that we need

to consider is the application of these microbes in the natural field condition. Several studies that were previously done is under controlled condition. So, the effectiveness of using beneficial microorganism under natural habitat is well desired. Besides this, in some extreme stress condition, rhizosphere microorganisms also copes with the stresses, so we need to look after this aspect also. When plants cope with adverse environmental situation, there is a combination of stressors that affects plant growth. Furthermore, most of studies have been carried out only under one stress condition. So, the effects of symbiotic microorganisms need to be explored under multiple stress conditions. To commercialize these microbes for future protection of plants needs more trial and more studies. Lastly, it can be concluded that symbiotic microorganisms hold an important place in providing plant stress tolerance which has the potential to ensure future food security.

## KEYWORDS

- **arbuscular mycorrhiza**
- **endophytes**
- **glomalin**
- **mycorrhiza-induced resistance**
- **rhizobacteria**
- **stress tolerance**

## REFERENCES

- Abbaspour, H., Saeidi-Sar, S., Afshari, H., Abdel-Wahhab, M., & Abdel-Wahhab, M. A., (2012). Tolerance of mycorrhiza infected pistachio (*Pistacia vera* L.) seedling to drought stress under glasshouse conditions. *J. Plant. Physiol.*, 169, 704–709.
- Abd El-Azeem, S. A. M., Elwan, M. W. M., Sung, J. K., & Ok, Y. S., (2012). Alleviation of salt stress in eggplant (*Solanum melongena* L.) by plant-growth-promoting rhizobacteria. *Commun. Soil Sci. Plant Anal.*, 43, 1303–1315.
- Ahmad, M., & Kibret, M., (2013). Mechanism and applications of plant growth promoting rhizobacteria: Current perspective. *J. King. Saud. Univ. Sci.*, 26, 1–20.
- Ahuja, I., De Vos, R. C. H., Bones, A. M., & Hall, R. D., (2010). Plant molecular stress responses face climate change. *Trends. Plant Sci.*, 15, 664–674.
- Al-Hmoud, G., & Al-Momany, A., (2017). Effect of four mycorrhizal products on squash plant growth and its effect on physiological plant elements. *Adv. Crop. Sci. Technol.*, 5, 1–6.



- Ali, S., Charles, T. C., & Glick, B. R., (2014). Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Plant Physiol. Bioch.*, 80, 160–167.
- Alizadeh, O., Zare, M., & Nasr, A. H., (2011). Evaluation effect of mycorrhiza inoculate under drought stress condition on grain yield of sorghum (*Sorghum bicolor*). *Adv. Environ. Biol.*, 5, 2361–1364.
- Al-Karaki, G. N., (2006). Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. *Sci. Hortic.*, 109, 1–7.
- Allen, M. F., (2011). Linking water and nutrients through the vadose zone: A fungal interface between the soil and plant systems. *J. Arid. Land.*, 3, 155–163.
- Apel, K., & Hirt, H., (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant. Biol.*, 55, 373–399.
- Arkhipova, T. N., Prinsen, E., Veselov, S. U., Martinenko, E. V., Melentiev, A. I., & Kudoyarova, G. R., (2007). Cytokinin producing bacteria enhance plant growth in drying soil. *Plant. Soil.*, 292, 305–315.
- Ashraf, M., Shahbaz, M., & Ali, Q., (2013). Drought-induced modulation in growth and mineral nutrients in canola (*Brassica napus* L.). *Pak. J. Bot.*, 45, 93–98.
- Audet, P., & Charest, C., (2007). Heavy metal phytoremediation from a meta-analytical perspective. *Environ. Pollut.*, 147, 231–237.
- Babu, A. G., Shea, P. J., Sudhakar, D., Jung, I. B., & Oh, B. T., (2015). Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal (loid)-contaminated mining site soil. *J. Environ. Manage.*, 151, 160–166.
- Baltruschat, H., Fodor, J., Harrach, B. D., Niemczyk, E., Barna, B., Gullner, G., Janeczko, A., et al., (2008). Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytol.*, 180, 501–510.
- Bao, X., Wang, Y., Li, S., & Olsson, P. A., (2019). Arbuscular mycorrhiza under water carbon-phosphorus exchange between rice and arbuscular mycorrhizal fungi under different flooding regimes. *Soil Biol. Biochem.*, 129, 169–177.
- Bárzana, G., Aroca, R., Paz, J. A., Chaumont, F., Martínez-Ballesta, M. C., Carvajal, M., & Ruiz-Lozano, J. M., (2012). Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Ann. Bot.*, 109, 1009–1017.
- Bennett, A. E., & Classen, A., (2020). Climate change influences mycorrhizal fungal–plant interactions, but conclusions are limited by geographical study bias. *Ecology*, 101, e02978.
- Berg, G., (2009). Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.*, 84, 11–18.
- Bernardo, L., Carletti, P., Badeck, F., Rizza, F., Morcia, C., Ghizzoni, R., Rouphael, Y., et al., (2019). Metabolomic responses triggered by arbuscular mycorrhiza enhance tolerance to water stress in wheat cultivars. *Plant. Physiol. Biochem.*, 137, 203–212.
- Bhattacharyya, P. N., & Jha, D. K., (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.*, 28, 1327–1350.
- Cameron, D. D., Neal, A. L., Van, W. S. A., & Ton, J., (2013). Mycorrhiza-induced resistance: More than the sum of its parts? *Trends Plant. Sci.*, 18, 539–545.
- Cawoy, H., Mariutto, M., Henry, G., Fisher, C., Vasilyeva, N., Thonart, P., Dommes, J., & Ongena, M., (2013). Plant defense stimulation by natural isolates of *Bacillus* depends on efficient surfactin production. *Mol. Plant Microbe Interact.*, 27, 87–100.

- Chadha, N., Mishra, M., Rajpal, K., Bajaj, R., Choudhary, D. K., & Varma, A., (2015). An ecological role of fungal endophytes to ameliorate plants under biotic stress. *Arch. Microbiol.*, 197, 869–881.
- Chakraborty, N., Chakraborty, N., Acharyya, P., & Acharya, K., (2021). Isolation, characterization and identification of novel broad-spectrum bacterial antagonist(s) to control *Fusarium* wilt of eggplant. *Physiologic. Mol. Plant Pathol.*, 116, 101711.
- Chen, C., Xin, K., Liu, H., Cheng, J., Shen, X., Wang, Y., & Zhang, L., (2017). *Pantoea alhagi*, a novel endophytic bacterium with the ability to improve growth and drought tolerance in wheat. *Sci. Rep.*, 7, 1–14.
- Chen, T., Johnson, R., Chen, S., Lv, H., Zhou, J., & Li, C., (2018). Infection by the fungal endophyte *Epichloë bromicola* enhances the tolerance of wild barley (*Hordeum brevisubulatum*) to salt and alkali stresses. *Plant Soil.*, 428, 353–370.
- Cherif, H., Marasco, R., Rolli, E., Ferjani, R., Fusi, M., & Soussi, A., (2015). Oasis desert farming selects environment-specific date palm root endophytic communities and cultivable bacteria that promote resistance to drought. *Env. Microbiol. Rep.*, 7, 668–678.
- Chu, X., Fu, J., Sun, Y., Xu, Y., Miao, Y., Xu, Y., & Hu, T., (2016). Effect of arbuscular mycorrhizal fungi inoculation on cold stress-induced oxidative damage in leaves of *Elymus nutans* griseb. *S. Afr. J. Bot.*, 104, 21–29.
- Coccina, A., Cavagnaro, T. R., Pellegrino, E. E., Ercoli, L., McLaughlin, M., & Watts-Williams, S. J., (2019). The mycorrhizal pathway of zinc uptake contributes to zinc accumulation in barley and wheat grain. *BMC Plant. Biol.*, 19, 133–147.
- Cohen, A. C., Bottini, R., Pontin, M., Berli, F. J., Moreno, D., & Boccanlandro, H., (2015). *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. *Physiol. Plantarum.*, 153, 79–90.
- Cramer, G. R., Urano, K., Delrot, S., Pezzotti, M., & Shinozaki, K., (2011). Effects of abiotic stress on plants: A systems biology perspective. *BMC Plant Biol.*, 11, 163.
- Creus, C. M., Sueldo, R. J., & Barassi, C. A., (1997). Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses. *Plant Physiol. Biochem.*, 35, 939–944.
- Daci, G., Ardekani, M., Rejali, F., Teimuri, S., & Miransari, M., (2009). Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. *J. Plant Physiol.*, 166, 217–225.
- Dastogeer, K. M. G., Chakraborty, A., Sarker, M. S. A., & Akter, M. A., (2020). Roles of fungal endophytes and viruses in mediating drought stress tolerance in plants. *Int. J. Agric. Biol.*, 24, 1497–1512.
- Dell'Amico, J., Torrecillas, A., Rodríguez, P., Morte, A., & Sánchez-Blanco, M. J., (2002). Responses of tomato plants associated with the arbuscular mycorrhizal fungus *Glomus clarum* during drought and recovery. *J. Agric. Sci.*, 138, 387–393.
- Diagne, N., Ngom, M., Djighaly, P. I., Fall, D., Hoher, V., & Svistoono, S., (2020). Roles of arbuscular mycorrhizal fungi on plant growth and performance: Importance in biotic and abiotic stressed regulation. *Diversity*, 12, 370.
- Egamberdieva, D., Wirth, S. J., Shurigin, V. V., Hashem, A., & Abdallah, E. F., (2017). Endophytic bacteria improve plant growth, symbiotic performance of chickpea (*Cicer arietinum* L.) and induce suppression of root rot caused by *Fusarium solani* under salt stress. *Front. Microbiol.*, 8, 1887–1899.

- Estrada, B., Aroca, R., Maathuis, F. J. M., Barea, J. M., & Ruiz-Lozano, J. M., (2013). Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. *Plant Cell Environ.*, 36, 1771–1782.
- Faber, B. A., Zasoski, R. J., Munns, D. N., & Shackel, K., (1991). A method for measuring hyphal nutrient and water uptake in mycorrhizal plants. *Can. J. Bot.*, 69, 87–94.
- Fougnies, L., Renciot, S., Müller, F., Plenchette, C., Prin, Y., De Faria, S. M., Bouvet, J. M., et al., (2006). Arbuscular mycorrhizal colonization and nodulation improve flooding tolerance in *Pterocarpus officinalis* Jacq. seedlings. *Mycorrhiza*, 17, 159–166.
- Friesen, M. L., Porter, S. S., Stark, S. C., Von, W. E. J., Sachs, J. L., & Martinez-Romero, E., (2011). Microbially mediated plant functional traits. *Annu. Rev. Ecol. Evol. Syst.*, 42, 23–46.
- Gadkar, V., & Rillig, M. C., (2006). The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. *FEMS. Microbiol. Lett.*, 263, 93–101.
- Gagné-Bourque, F., Mayer, B. F., Charron, J. B., Vali, H., Bertrand, A., & Jabaji, S., (2015). Accelerated growth rate and increased drought stress resilience of the model grass *Brachypodium distachyon* colonized by *Bacillus subtilis* B26. *PLoS One*, 10, e0130456.
- Ghanem, M. E., Albacete, A., Smigocki, A. C., Frebort, I., Pospisilova, H., Martinez-Andujar, C., Acosta, M., et al., (2011). Root-synthesized cytokinins improve shoot growth and fruit yield in salinized tomato (*Solanum lycopersicum* L.) plants. *J. Exp. Bot.*, 62, 125–140.
- Gill, S. S., & Tuteja, N., (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant. Physiol. Biochem.*, 48, 909–930.
- Glick, B. R., Cheng, Z., Czarny, J., Cheng, Z., & Duan, J., (2007). Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant. Pathol.*, 119, 329–339.
- Glick, B. R., Penrose, D. M., & Li, J., (1998). A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *J. Theor. Biol.*, 190, 3–68.
- Gond, S. K., Torres, M. S., Bergen, M. S., Helsel, Z., & White, J. F., (2015). Induction of salt tolerance and up-regulation of aquaporin genes in tropical corn by rhizobacterium *Pantoea agglomerans*. *Lett. Appl. Microbiol.*, 60, 392–399.
- González-Guerrero, M., Escudero, V., Saéz, Á., & Tejada-Jiménez, M., (2016). Transition metal transport in plants and associated endosymbionts: Arbuscular mycorrhizal fungi and rhizobia. *Front. Plant. Sci.*, 7, 1088.
- González-Teuber, M., Pozo, M. J., Muck, A., Svatos, A., Adame-Alvarez, R. M., & Heil, M., (2010). Glucanases and chitinases as causal agents in the protection of *Acacia* extrafloral nectar from infestation by phytopathogens. *Plant. Physiol.*, 152, 1705–1715.
- Gulyani, V., Kushwaha, H. R., & Kumar, P., (2018). Role of phytohormones in plant defense: Signaling and cross talk. In: Singh, A., & Singh, I. K., (eds.), *Molecular Aspects of Plant-Pathogen Interaction* (pp. 159–184). Springer Nature; Singapore.
- Guo, Y., Ni, Y., & Huang, J., (2010). Effects of rhizobium, arbuscular mycorrhiza and lime on nodulation, growth and nutrient uptake of lucerne in acid purplish soil in China. *Trop. Grasslands*, 44, 109–114.
- Hajiboland, R., Joudmand, A., Aliasgharzad, N., Tolrá, R., & Poschenrieder, C., (2019). Arbuscular mycorrhizal fungi alleviate low-temperature stress and increase freezing resistance as a substitute for acclimation treatment in barley. *Crop. Pasture Sci.*, 70, 218–233.
- Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M., (2013). Physiological, biochemical and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci.*, 14, 9643–9684.

- Hashem, A., Allah, E. A., Alqarawi, A. A., Wirth, S., & Egamberdieva, D., (2016). Comparing symbiotic performance and physiological responses of two soybean cultivars to arbuscular mycorrhizal fungi under salt stress. *Saudi J. Biol. Sci.*, 26, 38–48.
- Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I., (2010). Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann. Microbiol.*, 60, 579–598.
- Hilleary, R., & Gilroy, S., (2018). Systemic signaling in response to wounding and pathogens. *Curr. Opin. Plant Biol.*, 43, 57–62.
- Ilangumaran, G., & Smith, D. L., (2017). Plant growth promoting rhizobacteria in amelioration of salinity stress: A systems biology perspective. *Front. Plant Sci.*, 8, 1768.
- Jaemsaeng, R., Jantasuriyarat, C., & Thamchaipenet, A., (2018). Molecular interaction of 1-aminocyclopropane-1-carboxylate deaminase (ACCD)-producing endophytic *Streptomyces* sp. GMKU 336 towards salt-stress resistance of *Oryza sativa* L. cv. KDML105. *Sci. Rep.*, 8, 1950.
- Jiang, Q. Y., Zhuo, F., Long, S. H., Zhao, H. D., Yang, D. J., Ye, Z. H., Li, S. S., & Jing, Y. X., (2016). Can arbuscular mycorrhizal fungi reduce Cd uptake and alleviate Cd toxicity of *Lonicera japonica* grown in Cd-added soils? *Sci. Rep.*, 6, 21805.
- Jin, H. Q., Liu, H. B., Xie, Y. Y., Zhang, Y. G., Xu, Q. Q., & Mao, L. J., (2017). Effect of the dark septate endophytic fungus *Acrocalymma vagum* on heavy metal content in tobacco leaves. *Symbiosis*, 74, 89–95.
- Joe, M. M., Islam, M. R., Karthikeyan, B., Bradeepa, K., & Sa, T., (2012). Resistance responses of rice to rice blast fungus after seed treatment with the endophytic *Achromobacter xylosoxidans* AUM54 strains. *Crop Prot.*, 42, 141–148.
- Kaldorf, M., Kuhn, A., Schröder, W., Hildebrandt, U., & Bothe, H., (1999). Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. *J. Plant. Physiol.*, 154, 718–728.
- Kasim, W. A., Osman, M. E., Omar, M. N., Abd El-Daim, I. A., Bejai, S., & Meijer, J., (2013). Control of drought stress in wheat using plant growth-promoting bacteria. *J. Plant Growth Regul.*, 32, 122–130.
- Kaya, C., Higgs, D., Kirnak, H., & Tas, I., (2003). Mycorrhizal colonization improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions. *Plant. Soil.*, 253, 287–292.
- Kelkar, T. S., & Bhalerao, S. A., (2013). Beneficiary effect of arbuscular mycorrhiza to *Trigonella Foenum-Graceum* in contaminated soil by heavy metal. *Res. J. Recent Sci.*, 2, 29–32.
- Kim, S. J., Eo, J. K., Lee, E. H., Park, H., & Eom, A. H., (2017). Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. *Mycobiology*, 45, 20–24.
- Kumar, A., Patel, J. S., Meena, V. S., & Ramteke, P. W., (2019). Plant growth promoting rhizobacteria: Strategies to improve abiotic stresses under sustainable agriculture, *J. Plant Nutr.*, 42, 1–15.
- Kumar, M., (2013). Crop plants and abiotic stresses. *J. Biomol. Res. Ther.*, 3, 1.
- Lata, R., Chowdhury, S., Gond, S. K., & White, J. F., (2018). Induction of abiotic stress tolerance in plants by endophytic microbes. *Lett. Appl. Microbiol.*, 66, 268–276.
- Laxa, M., Liebthal, M., Telman, W., Chibani, K., & Dietz, K. J., (2019). The role of the plant antioxidant system in drought tolerance. *Antioxidants*, 8, 94–125.
- Li, X., & Christie, P., (2001). Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. *Chemosphere*, 42, 201–207.

- Li, Z., Wu, N., Meng, S., Wu, F., & Liu, T., (2020). Arbuscular mycorrhizal fungi (AMF) enhance the tolerance of *Euonymus maackii* Rupr. at a moderate level of salinity. *PLoS One*, 15, e0231497.
- Lichtenthaler, H. K., (1998). *The Stress Concept in Plants: An Introduction* (Vol. 851, pp. 187–198). Annals of the New York Academy of Sciences.
- Lindstrom, J. T., & Belanger, F. C., (1994). Purification and characterization of an endophytic fungal proteinase that is abundantly expressed in the infected host grass. *Plant. Physiol.*, 106, 7–16.
- Mahaffee, W. F., & Kloepper, J. W., (1997). Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). *Microbial. Ecol.*, 34, 210–223.
- Majeed, A., Abbasi, M. K., Hameed, S., Imran, A., & Rahim, N., (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Front. Microbiol.*, 6, 198.
- Malinowski, C. P., & Beleskey, D. P., (2000). Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. *Crop. Sci.*, 40, 923–940.
- Massad, T. J., Dyer, L. A., & Vega, C. G., (2012). Costs of defense and a test of the carbon–nutrient balance and growth–differentiation balance hypotheses for two co-occurring classes of plant defense. *PLoS One*, 7, e47554.
- Mathur, S., & Jajoo, A., (2020). Arbuscular mycorrhizal fungi protect maize plants from high temperature stress by regulating photosystem II heterogeneity. *Ind. Crop. Prod.*, 143, 111934.
- Mattos, K. A., Pádua, V. L. M., Romeiro, A., Hallack, L. F., Neves, B. C., Ulisses, T. M., Barros, C. F., et al., (2008). Endophytic colonization of rice (*Oryza sativa* L.) by the diazotrophic bacterium *Burkholderia kururiensis* and its ability to enhance plant growth. *An. Acad. Bras Ciênc.*, 80, 477–493.
- Maya, M. A., & Matsubara, Y. I., (2013). Influence of arbuscular mycorrhiza on the growth and antioxidative activity in cyclamen under heat stress. *Mycorrhiza*, 23, 381–390.
- Mayak, S., Tirosh, T., & Glick, B. R., (2004). Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. *Plant Sci.*, 166, 525–530.
- Mei, C., & Flinn, B. S., (2010). The use of beneficial microbial endophytes for plant biomass and stress tolerance improvement. *Recent Pat. Biotechnol.*, 4, 81–95.
- Mongkolthanaruk, W., (2012). Classification of *Bacillus* beneficial substances related to plants, humans and animals. *J. Microbiol. Biotechnol.*, 22, 1597–1604.
- Muhammad, H., Sumera, A. K., Abdul, L. K., Gauhar, R., Youn-Ha, K., Ilyas, I., Javid, H., Eun-Young, S., & In-Jung, L., (2010). Gibberellin production and plant growth promotion from pure cultures of *Cladosporium* sp. MH-6 isolated from cucumber (*Cucumis sativus* L.). *Mycologia*, 102(5), 989–995.
- Nadeem, S. M., Maqshoof, A., Zahir, Z. A., Javaid, A., & Ashraf, M., (2013). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.*, 32, 429–448.
- Nagata, S., Yamaji, K., Nomura, N., & Ishimoto, H., (2015). Root endophytes enhance stress-tolerance of *Cicuta virosa* L. growing in a mining pond of eastern Japan. *Plant. Spec. Biol.*, 30, 116–125.

- Nakmee, P. S., Techapinyawat, S., & Ngamprasit, S., (2016). Comparative potentials of native arbuscular mycorrhizal fungi to improve nutrient uptake and biomass of *Sorghum bicolor* Linn. *Agric. Nat. Resour.*, 50, 173–178.
- Nanda, S., Rout, E., & Joshi, R. K., (2016). Curcuma longa mitogen-activated protein kinase 6 (CIMPK6) stimulates the defense response pathway and enhances the resistance to necrotrophic fungal infection. *Plant. Mol. Biol. Report.*, 34, 886–898.
- Naveed, M., Mitter, B., Reichenauer, T. G., Wieczorek, K., & Sessitsch, A., (2014). Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. *Environ. Exp. Bot.*, 97, 30–39.
- Naya, L., Ladrera, R., Ramos, J., Gonzalez, E. M., Arrese-Igor, C., Minchin, F. R., & Becana, M., (2007). The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. *Plant Physiol.*, 144, 1104–1114.
- Nezhadahmadi, A., Prodhan, Z. H., & Faruq, G., (2013). Drought tolerance in wheat. *Scientific World Journal*, 2013, 1–12.
- Nguvo, K. J., & Gao, X., (2019). Weapons hidden underneath: Bio-control agents and their potentials to activate plant induced systemic resistance in controlling crop *Fusarium* diseases. *J. Plant. Dis. Prot.*, 126, 177–190.
- Nia, S. H., Zarea, M. J., Rejali, F., & Varma, A., (2012). Yield and yield components of wheat as affected by salinity and inoculation with *Azospirillum* strains from saline or non-saline soil. *J. Saudi Soc. Agric. Sci.*, 11, 113–121.
- Noctor, G., & Foyer, C. H., (1998). Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 49, 249–279.
- Olson, P. A., Thingstrub, I., Jakobsen, I., & Baath, E., (1999). Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. *Soil Biol. Biochem.*, 31, 1879–1887.
- Ortiz, N., Armada, E., Duque, E., Roldan, A., & Azcón, R., (2015). Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. *J. Plant. Physiol.*, 174, 87–96.
- Pan, X., Qin, Y., & Yuan, Z., (2018). Potential of a halophyte-associated endophytic fungus for sustaining Chinese white poplar growth under salinity. *Symbiosis.*, 76, 109–116.
- Pandey, V., Ansari, M. W., Tula, S., Yadav, S., Sahoo, R. K., Shukla, N., Bains, G., et al., (2016). Dose dependent response of *Trichoderma harzianum* in improving drought tolerance in rice genotypes. *Planta*, 243, 1251–1264.
- Pedranzani, H., Rodríguez-Rivera, M., Gutierrez, M., Porcel, R., Hause, B., & Ruiz-Lozano, J. M., (2015). Arbuscular mycorrhizal symbiosis regulates physiology and performance of *Digitaria eriantha* plants subjected to abiotic stresses by modulating antioxidant and jasmonate levels. *Mycorrhiza.*, 26, 141–152.
- Rakshapal, S., Sumit, K. S., Rajendra, P. P., & Alok, K., (2013). Technology for improving essential oil yield of *Ocimum basilicum* L. (sweet basil) by application of bioinoculant colonized seeds under organic field conditions. *Ind. Crops Prod.*, 45, 335–342.
- Redman, R. S., Sheehan, K. B., Stout, R. G., Rodriguez, R. J., & Henson, J. M., (2002). Thermotolerance generated by plant/ fungal symbiosis. *Science*, 298, 1581.
- Ren, A., Gao, Y., Zhang, L., & Xie, F., (2006). Effects of cadmium on growth parameters of endophyte-infected endophyte-free ryegrass. *J. Plant Nutr. Soil Sci.*, 169, 857–860.
- Rodriguez, R. J., Henson, J., Volkenburgh, E. V., Hoy, M., Wright, L., Beckwith, F., Kim, Y. O., & Redman, R. S., (2008). Stress tolerance in plants via habitat-adapted symbiosis. *ISME J.*, 2, 404–416.

- Rodriguez, R. J., White, J. F., Arnold, A. E., & Redman, R. S., (2009). Fungal endophytes: Diversity and functional roles. *New. Phytol.*, 182, 314–330.
- Rojas-Tapias, D., Moreno-Galvan, A., Pardo-Díaz, S., Obando, M., Rivera, D., & Bonilla, R., (2012). Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). *Appl. Soil Ecol.*, 61, 264–272.
- Rozpądek, P., Węzowicz, K., Nosek, M., Wazny, R., Tokarz, K., Lembicz, M., Miszański, Z., & Turnau, K., (2015). The fungal endophyte *Epichloë typhina* improves photosynthesis efficiency of its host orchard grass (*Dactylis glomerata*). *Planta*, 242, 1025–1035.
- Safronova, V. I., Stepanok, V. V., Engqvist, G. L., Alekseyev, Y. V., & Belimov, A. A., (2006). Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. *Biol. Fertil. Soils*, 42, 267–272.
- Saravanan, V. S., Madhaiyan, M., Osborne, J., Thangaraju, M., & Sa, T. M., (2008). Ecological occurrence of *Gluconacetobacter diazotrophicus* and nitrogen-fixing *Acetobacteraceae* members: Their possible role in plant growth promotion. *Microbial. Ecol.*, 55, 130–140.
- Sarma, M. V. R. K., Kumar, V., Saharan, K., Srivastava, R., Sharma, A. K., Prakash, A., Sahai, V., & Bisaria, V. S., (2011). Application of inorganic carrier-based formulations of fluorescent pseudomonads and *Piriformospora indica* on tomato plants and evaluation of their efficacy. *J. Appl. Microbiol.*, 111, 456–466.
- Shi, C., Yan, P., Li, J., Wu, H., Li, Q., & Guan, S., (2014). Biocontrol of *Fusarium graminearum* growth and deoxynivalenol production in wheat kernels with bacterial antagonists. *Int. J. Environ. Res. Public Health*, 11, 1094–1105.
- Shiomi, H. F., Silva, H. S. A., Melo, I. S. D., Nunes, F. V., & Bettiol, W., (2006). Bioprospecting endophytic bacteria for biological control of coffee leaf rust. *Sci. Agric.*, 63, 32–39.
- Singh, R. P., & Jha, P. N., (2016). Alleviation of salinity-induced damage on wheat plant by an ACC deaminase-producing halophilic bacterium *Serratia* sp SL- 12 isolated from a salt lake. *Symbiosis*, 69, 101–111.
- Solís-Rodríguez, U. R. J., Ramos-Zapata, J., Hernández-Cuevas, L., Salinas-Peba, L., & Guadarrama, P., (2020). Arbuscular mycorrhizal fungi diversity and distribution in tropical low flooding forest in Mexico. *Mycol. Prog.*, 19, 195–204.
- Spagnoletti, F. N., Cornero, M., Chiochio, V., Lavado, R. S., & Roberts, I. N., (2020). Arbuscular mycorrhiza protects soybean plants against *Macrophomina phaseolina* even under nitrogen fertilization. *Eur. J. Plant. Pathol.*, 156, 839–849.
- Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., Bonito, G., et al., (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia.*, 108, 1028–1046.
- Srivastava, S., Verma, P. C., Chaudhry, V., Singh, N., Abhilash, P. C., Kumar, K. V., Sharma, N., & Singh, N., (2013). Influence of inoculation of arsenic-resistant *Staphylococcus arlettae* on growth and arsenic uptake in *Brassica juncea* (L.) Czern. Var. R-46. *J. Hazard Mater.*, 262, 1039–1047.
- Su, F., Jacquard, C., Villalume, S., Michel, J., Rabenoelina, F., Clement, C., Barka, E. A., et al., (2015). *Burkholderia phytofirmans* PsJN reduces the impact of freezing temperatures on photosynthesis in *Arabidopsis thaliana*. *Front. Plant Sci.*, 6, 810.
- Suarez, R., Wong, A., Ramirez, M., Barraza, A., Orozco, M. C., & Cevallos, M. A., (2008). Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate synthase in rhizobia. *Mol. Plant-Microbe. In.*, 21, 958–966.

- Subramanian, P., Mageswari, A., Kim, K., Lee, Y., & Sa, T., (2015). Psychrotolerant endophytic *Pseudomonas* sp. strains OB155 and OS261 induced chilling resistance in tomato plants (*Solanum lycopersicum* mill.) by activation of their antioxidant capacity. *Mol. Plant Microb. Int.*, 28, 1073–1081.
- Suman, A., Gaur, A., Shrivastava, A. K., & Yadav, R. L., (2005). Improving sugarcane growth and nutrient uptake by inoculating *Gluconacetobacter diazotrophicus*. *Plant. Growth. Regul.*, 47, 155–162.
- Sun, C., Johnson, J. M., Cai, D., Sherameti, I., Oelmeuller, R., & Lou, B., (2010). *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *J. Plant Physiol.*, 167, 1009–1017.
- Suzuki, S., He, Y., & Oyaizu, H., (2003). Indole-3-acetic acid production in *Pseudomonas fluorescens* HP72 and its association with suppression of creeping bentgrass brown patch. *Curr. Microbiol.*, 47, 138–143.
- Theocharis, A., Clement, C., & Barka, E. A., (2012). Physiological and molecular changes in plants grown at low temperatures. *Planta*, 235, 1091–1105.
- Tian, C., Feng, G., Li, X., & Zhang, F., (2004). Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. *Appl. Soil Ecol.*, 26, 143–148.
- Timmusk, S., & Wagner, E. G. H., (1999). The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: A possible connection between biotic and abiotic stress responses. *Mol. Plant-Microbe Interact.*, 12, 951–959.
- Ting, A. S. Y., Mah, S. W., & Tee, C. S., (2012). Evaluating the feasibility of induced host resistance by endophytic isolate *Penicillium citrinum* BTF08 as a control mechanism for *Fusarium* wilt in banana plantlets. *Biol. Control.*, 61, 155–159.
- Verma, H., Kumar, D., Kumar, V., Kumari, M., Singh, S. K., Sharma, V. K., Droby, S., et al., (2021). The potential application of endophytes in management of stress from drought and salinity in crop plants. *Microorganisms*, 9, 1729.
- Vivas, A., Marulanda, A., Ruiz-Lozano, J. M., Barea, J. M., & Azcon, R., (2003). Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. *Mycorrhiza*, 13, 249–256.
- Vos, C., Tesfahun, A., Panis, B., De Waele, D., & Elsen, A., (2012). Arbuscular mycorrhizal fungi induce systemic resistance in tomato against the sedentary nematode *Meloidogyne incognita* and the migratory nematode *Pratylenchus penetrans*. *Appl. Soil Ecol.*, 61, 1–6.
- Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R., (2007). Heat tolerance in plants: An overview. *Environ. Exp. Bot.*, 61, 199–223.
- Wang, C., Wang, C., Gao, Y. L., Wang, Y. P., & Guo, J. H., (2016). A consortium of three plant growth-promoting rhizobacterium strains acclimates *Lycopersicon esculentum* and confers a better tolerance to chilling stress. *J. Plant. Growth. Regul.*, 35, 54–64.
- Wang, F., (2017). Occurrence of arbuscular mycorrhizal fungi in mining-impacted sites and their contribution to ecological restoration: Mechanisms and applications. *Crit. Rev. Environ. Sci. Technol.*, 47, 1–57.
- Waqas, M., Khan, A. L., Kamran, M., Hamayun, M., Kang, S. M., Kim, Y. H., & Lee, I. J., (2012). Endophytic fungi produce gibberellins and indoleacetic acid and promote host-plant growth during stress. *Molecules*, 17, 10754–10773.



- Wiewiora, B., Zurek, G., & Panka, D., (2015). Is the vertical transmission of *Neotyphodium lolii* in perennial ryegrass the only possible way to the spread of endophytes? *PLoS One*, 10, 0117231.
- Wu, Z., Peng, Y., Guo, L., & Li, C., (2014). Root colonization of encapsulated *Klebsiella oxytoca* Rs-5 on cotton plants and its promoting growth performance under salinity stress. *Eur. J. Soil Biol.*, 60, 81–87.
- Xu, L., Wang, A., Wang, J., Wei, Q., & Zhang, W., (2017). *Piriformospora indica* confers drought tolerance on *Zea mays* L. through enhancement of antioxidant activity and expression of drought-related genes. *Crop J.*, 5, 251–258.
- Xu, Z., Jiang, Y., Jia, B., & Zhou, G., (2016). Elevated-CO<sub>2</sub> response of stomata and its dependence on environmental factors. *Front. Plant Sci.*, 7, 657.
- Yadav, S. K., (2010). Cold stress tolerance mechanisms in plants. A review. *Agron. Sustain. Dev.*, 30, 515–527.
- Yooyongwech, S., Phaukinsang, N., Cha-Um, S., & Supaibulwatana, K., (2013). Arbuscular mycorrhiza improved growth performance in *Macadamia tetraphylla* L. grown under water deficit stress involves soluble sugar and proline accumulation. *Plant Growth Regul.*, 69, 285–293.
- Zarea, M. J., Hajinia, S., Karimi, N., Goltapeh, E. M., Rejali, F., & Varma, A., (2012). Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl. *Soil Biol. Biochem.*, 45, 139–146.
- Zhang, F., Zhang, H., Wang, G., Xu, L., & Shen, Z., (2009). Cadmium-induced accumulation of hydrogen peroxide in the leaf apoplast of *Phaseolus aureus* and *Vicia sativa* and the roles of different antioxidant enzymes. *J. Hazard. Mater.*, 168, 76–84.
- Zhang, Y., Yu, X., Zhang, W., Lang, D., Zhang, X., Cui, G., & Zhang, X., (2019). Interactions between endophytes and plants: Beneficial effect of endophytes to ameliorate biotic and abiotic stresses in plants. *J. Plant Biol.*, 62, 1–13.
- Zhou, X. R., Dai, L., Xu, G. F., & Wang, H. S., (2021). A strain of *Phoma* species improves drought tolerance of *Pinus tabulaeformis*. *Sci. Rep.*, 11, 7637.
- Zhu, X. C., Song, F. B., Liu, S. Q., Liu, T. D., & Zhou, X., (2012). Arbuscular mycorrhizae improves photosynthesis and water status of *Zea mays* L. under drought stress. *Plant Soil Environ.*, 58, 186–191.

**PART II**

**Priming and Genetic Engineering**



# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

## CHAPTER 4

---

# Role of Hydropriming and Magneto-Priming in Developing Stress Tolerance

LEKSHMY SATHEE,<sup>1\*</sup> MEENAKSHI THAKUR,<sup>2</sup> RAVPREET KAUR,<sup>3</sup>  
SUNITA KATARIA,<sup>4</sup> ANJALI ANAND,<sup>1</sup> DALVEER SINGH,<sup>3</sup> and  
SHAILESH TRIPATHI<sup>3</sup>

<sup>1</sup>*Division of Plant Physiology, ICAR–Indian Agricultural Research Institute, New Delhi – 110012, India*

<sup>2</sup>*College of Horticulture and Forestry, Dr. Y.S. Parmar University of Horticulture and Forestry, Neri, Himachal Pradesh, India*

<sup>3</sup>*Division of Genetics, ICAR–Indian Agricultural Research Institute, New Delhi, India*

<sup>4</sup>*School of Biochemistry, DAVV, Indore, Madhya Pradesh, India*

*\*Corresponding author. E-mail: lekshmyrnair@gmail.com*

---

## ABSTRACT

Seed priming is a highly effective and low-cost seed enhancement technique to improve germination, early vigor, growth, and yield of crop plants. It reduces the time of seedling emergence and helps in the establishment of a uniform crop stand. Several priming agents, such as water, inorganic salts, sugars, micronutrients, plant hormones, and physical treatments with ionizing/nonionizing radiation are used as seed priming agents. Seed priming also confers plants with tolerance to various abiotic stresses. Primed seeds invigorate the seed metabolism through the repair and synthesis of nucleic acids and proteins. In hydro-priming, the seeds are soaked in water for a well-defined period of time followed by drying to their original moisture level.

Conversely, magneto-priming is a non-ionizing, physical energy, dry seed treatment that evades the hydration and dehydration cycle. Both the priming techniques can alleviate the abiotic stress during seedling emergence and crop establishment stage, through an array of processes involving upregulation of various signaling pathways and antioxidative systems leading to production of stress-responsive “savior metabolites.” The evocation of stress tolerance mechanism is manifested at the plant level as synchrony in seed germination, early vigor, increased biomass, and yield. The plants can also respond to reproductive stage stress exposures by using the stress memory response. In this chapter, we will discuss the various mechanisms governing the stress response under different abiotic stresses in hydro- and magneto-primed seeds.

## **4.1 INTRODUCTION**

Plants are exposed to various abiotic factors such as drought, high salinity, submergence, and extreme temperature, etc., leading to a substantial loss in crop yield. The exposure to these stress signal leads to activation of different defense mechanisms, stress signaling, metabolite production, etc., resulting in physiological, metabolic, and biochemical changes in plants (Xiong & Zhu, 2002). These alterations may affect plant performance, yield, metabolism, and oxidative stress (Jisha et al., 2013). Various efforts have been adapted to develop plants that can withstand stresses and show minimal pre- and post-harvest losses in limited agricultural resources (Gust et al., 2010; Hasanuzzaman & Fotopoulos, 2019). Uniform seed germination, early seedling growth, and uniform plant stand (Hasanuzzaman & Fotopoulos, 2019) determines yield and plant response to stresses.

Seed priming is an effective method to strengthen the defense line of crop plants by imparting stress tolerance. Seed priming improves seed germination and crop stand. In general, seed priming is a physiological procedure of seed hydration followed by drying that enhances the pre-germinative metabolic process for faster germination, better growth, and production under normal as well as stressed conditions. It is a promising low-cost technique to induce germination, increase growth, and thus the productive ability of crops. In this process, different priming agents such as water, sugars, nutrients, hormones, micronutrients, organic sources, and inorganic salts, etc., are applied to regulate hydration level and activate germinative metabolic activities without radical protrusion (Hussain et al., 2015, 2018; Lutts et al., 2016; Zheng et al., 2016). The abiotic cues prompt warning signals to enhance the plant defense

mechanisms that change plant transcriptional, biochemical, physiological, and epigenetic levels upon stimulus perception (Anderson et al., 2018; Mauch-Mani et al., 2017). This is known as the priming phase which is followed by a post-challenge primed phase, where the plant shows a faster and stronger defense response upon subsequent challenge and has better stress tolerance. The substances released as an effect of priming are used to generate high-energy compounds essential for the germination of seedlings (Renugadevi & Vijayageetha, 2013). The result is activation of different enzymes, production of metabolites, protein synthesis, improved antioxidant repair mechanism and biochemical cell repair systems. Overall seed priming promotes germination rate, seed vigor, photosynthetic efficiency, root length, biomass production along with efficient performance under an extensive range of environments and more than a few agronomic and economic advantages. Some of the other benefits are efficient release from thermo- and photo-dormancy, improved water use efficiency, early maturity, better nutrient uptake, and high yield (Hill et al., 2008; Hussain et al., 2016). Better oxidative defense mechanisms have emerged from primed seeds acquired with enhanced levels of superoxide dismutase (SOD), glutathione reductase (GR), ascorbic acid (AsA), catalase (CAT), and peroxidases (POD), late embryogenesis abundant protein (LEA), dehydrins (DHN), and aquaporins (AQP) (Blokhina, 2003; Chen et al., 2013; Fashui, 2002; Wechsberg et al., 1995). Many studies have reported better crop establishment, uniform germination with advanced plant growth and stress resistance in seedlings, therefore, the productivity of field crops (Farooq et al., 2005, 2006a, b, 2007, 2008, 2010; Muhammad Farooq et al., 2008, 2006a, b). Seed priming is a durable process that can be maintained and transmitted from one generation to another, thus signifying a type of immunological memory in plants (Lutts et al., 2016; Mauch-Mani et al., 2017). However, different factors attribute to priming, such as plant species, priming media, storage conditions, temperature, light, seed characteristics, priming duration, and aeration.

## **4.2 MAGNETO-PRIMING INDUCED CHANGES IN PLANTS UNDER VARIOUS ABIOTIC STRESSES**

Magneto-priming is dry seed priming technique that omits the hydration and dehydration cycle which is inherent for wet priming treatments and allows storage of seeds at room temperature (Bhardwaj et al., 2012; Kataria & Jain, 2019; Kataria et al., 2019; Thakur et al., 2019). The effect of the magnetic field, first reported in plants by Krylov & Tarakanova (1960), has received

considerable attention in the recent past. It is based on the principle of biophysics that shows the effect of oscillating and low-frequency magnetic fields without heating the biological tissues. Pre-treatment of seeds/seedlings with magnetic field is gaining importance in applied agriculture as magnetic treatment improves the early vigor and initial stages of growth in plants (Nyakane et al., 2019; Jin et al., 2019). Positive effects on increase in percentage and rate of germination with improved seedling vigor have been reported in various crops, e.g., wheat (*Triticum aestivum*; Rathod & Anand, 2012), barley (*Hordeum vulgare*; Shabrangy et al., 2021), mungbean (*Vigna radiata*; Huang & Wang, 2008), sunflower (*Helianthus annuus*; Vashisth & Nagarajan, 2010), rice (*Oryza sativa*; Florez et al., 2004), chickpea (*Cicer arietinum*; Thomas et al., 2010), cucumber (*Cucumis sativus*; Bhardwaj et al., 2012), cherry tomato (Gupta et al., 2015), pea (Bhardwaj et al., 2016), soybean (*Glycine max*) and maize (*Zea mays*; Anand et al., 2012; Kataria et al., 2017a, b, 2019, 2020a, b), tomato (*Solanum lycopersicum*; Anand et al., 2019) and onion (*Allium cepa*; Thakur et al., 2020). The stimulatory response induced by magneto-priming is sustained till the maturity stage as manifested by improved plant growth, leaf area, photosynthesis, biomass, and crop yield (Kataria et al., 2019, 2020b, 2021). The protein patterns on SDS gel showed that leaves of soybean from static magnetic field (SMF)-primed seeds had higher band intensities of the large subunit (53 kDa) and small subunit (14 kDa) of Rubisco (Shine et al., 2012). Transcript abundance of large subunit of Rubisco and carbonic anhydrase (CA) genes were observed in leaves of soybean plants from SMF-primed seeds (Patel, 2020). In addition, proteomic analysis of SMF treated seeds of barley (Shabrangy et al., 2021) revealed that proteins carrying iron in their structure, with metal ion binding function, and those involved in electron transfer chain decreased compared to untreated seeds. Oscillation of metal ions by MFA exposure reduced the proteins with metal ion binding function, while the antioxidant system was upregulated due to the rise in cellular ROS levels. Total of 41 shoot specific proteins were identified as shoot tissue responded more than roots. The biological processes that were up regulated by magnetic field included carbohydrate metabolism, oxidation-reduction reactions and redox homeostasis (Shabrangy et al., 2021). Hormonal regulation of improved root growth was observed in studies with varying intensities and direction of magnetic field. Magnetic field in parallel with gravity vector increased cell division in the meristematic tissue due to higher auxin content which was regulated by expression of genes of auxin signaling pathways in the root tips (Jin et al., 2019). All these studies emphasize that magneto-priming can

be used as a feasible and chemical free solution to address crop productivity under non-stress and stressed environments like drought, salinity, UV B radiation, nutrient deficiency, and heavy metal toxicity, etc. (Mirdha et al., 2016; Razmjoo & Alinian, 2017; Baghel et al., 2018; Hozayn et al., 2018; Kataria & Jain, 2019; Islam et al., 2020; Kataria et al., 2020a, b, 2021; Fatima et al., 2020; Prajapati et al., 2020; Sarraf et al., 2020).

### **4.3 MAGNETO-PRIMING FOR IMPROVING PLANT PERFORMANCE UNDER ABIOTIC STRESSES**

Magneto-priming of the seeds has helped in improving the abiotic stress tolerance which the crop experiences pre- and post-germination. It becomes more relevant as remedies for early plant growth under soil-related limitations help in crop establishment that may be protected from later events of stress, like high/cold temperatures, by other strategies. We have discussed in detail the beneficial effects of magneto-priming towards various abiotic stresses under the different subheadings.

#### **4.2.1 SALT STRESS**

Salinity adversely affects seed germination and early seedling growth leading to reduced productivity in crops (Nawaz et al., 2012; Kataria & Verma, 2018). It lowers the osmotic potential, prevents water uptake and/or causes  $\text{Na}^+$  toxicity that prevents successful germination of the seeds (Khajeh-Hosseini et al., 2003). Similar to other abiotic stresses, ROS mediated oxidative stress results in malfunctioning of the cellular machinery (Ozgur et al., 2013; Kataria & Verma, 2018) by harming the biomolecules such as proteins, lipids, and nucleic acids (Gill & Tuteja, 2010). Application of compatible solutes *via* seed priming was observed to enhance salinity tolerance (Hare et al., 1998; Hasegawa et al., 2000; Sen & Puthur, 2020) due to accumulation of stress-induced compounds like free proline and malondialdehyde (MDA); antioxidant enzymes such as SOD, CAT, peroxidase (POD); ionic homeostasis and hormone regulation (Iqbal & Ashraf, 2010; Sen & Puthur, 2020). El-Yazied et al. (2011) observed that magneto-priming of tomato seeds resulted in a significant increase in the germination percentage and reduced the time needed for germination. This was validated in another study on chickpea and barley where magneto-priming had the potential to overcome the adverse effects of salinity at germination and early seedling growth stage (Thomas et al., 2013; Hozayn et al., 2018). Germination tolerance index



increased in SMF (200 mT for 1 h) pretreated maize seeds as compared to untreated seeds under 50 mM NaCl stress (Baghel et al., 2019). SMF helped in reducing the oxidative damage, owing to the up-regulation of antioxidant enzymes in stressed plants (Sarraf et al., 2020). The seedlings derived from magnetic field treated seeds exhibit an increased stem length, stem diameter, leaf area, and fresh and dry weight under saline conditions (El-Yazied et al., 2011). Baghel et al. (2016) indicated that SMF (200 milliTesla (mT) for 1 h) pre-treatment significantly increased number and weight of root nodules, photosynthetic efficiency, biomass, and yield of soybean plants in non-saline and saline conditions in comparison to untreated seeds. The content of leghe-moglobin and hemichrome also improved in the root nodules that indicated that the nodules remained active under salt stress. Seeds of other crops such as soybean and maize also responded to SMF pre-treatment by alleviating the salt-induced adverse effects on growth and yield as a result of more active reaction centers and higher efficiency of electron transport and faster reduction of electron acceptors in the photosynthetic pathway (Baghel et al., 2016, 2019; Kataria & Jain, 2019). SMF pre-treatment of the seeds eliminates the need for diverting the metabolic energy towards the detoxification of ROS produced under salt stress (Kataria & Jain, 2019). Kataria et al. (2019) reported that static magnetic field treatment (200 mT for 1 h) lowers the production of hydrogen peroxide ( $H_2O_2$ ), antioxidants (dehydroascorbic acid (DHA), and ascorbic acid (AsA)) and activities of antioxidant enzymes (SOD, POD, APX, GR) along with higher levels of  $\alpha$ -tocopherol in soybean leaves to facilitate higher growth, photosynthesis, and yield under salinity stress. Magnetoprimered seeds also showed higher uptake of water in soybean and maize and lower  $Na^+/K^+$  ratio in wheat under saline conditions (Kataria et al., 2017a, b; Rathod & Anand, 2016). Rathod & Anand (2016) reported that the sodium exclusion in the tissues of plants from magnetoprimered wheat seeds may be responsible for enhancing the salt tolerance. Pulsed magnetic field treatment had a positive effect on the regeneration of soybean calli under salt stressed condition by enhancing primary and secondary metabolite production (Radhakrishnan et al., 2012; Radhakrishnan & Kumari, 2013). Recently, Kataria et al. (2020a) identified the role of nitrate reductase (NR) dependent nitric oxide (NO) production in magnetoprimered induced tolerance towards salt stress in soybean during early seedling growth. They found that NO scavenger (2-[4-carboxyphenyl]-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, CPTIO), inhibitors of enzymes for NO synthesis, NO synthase (N-nitro-L-Arg-methyl ester, L-NAME) or NR (sodium tungstate, ST) and of NADPH oxidase (diphenylene iodonium (DPI)) significantly

decreased the SMF-induced seed germination, seedling growth, activities of  $\alpha$ -amylase, protease, and NR, ROS, and NO content in roots of soybean seedlings. Although the percentage of reduction in NO and NR was higher with ST treatment compared to LNAME in seedlings of SMF-primed seeds, the NO donor (sodium nitroprusside (SNP)) promoted all the parameters in saline and non-saline condition.

#### **4.2.2 DROUGHT STRESS**

Water stress can also potentially affect seed germination and early seedling growth (Ahmad et al., 2009). In maize, magneto-priming helped the seedlings to withstand moisture stress due to improved soil-water relations (Anand et al., 2012). Javed et al. (2011) found that SMF (100 and 150 mT for 10 min) was effective in alleviating the drought-induced adverse effects on maize growth by improving photosynthetic efficiency through photochemical and non-photochemical quenching. Selim & El-Nady (2011) reported that magnetic field treated tomato seeds resulted in stimulation of plant growth, cambium differentiation activity, thickness of mesophyll tissue, water uptake, proline concentration and photosynthetic pigments in response to drought stress. Improvement in chlorophyll and carotenoids and decreased SOD, POX, APX, and CAT activities under drought stress was also observed by Sen & Alikamanoglu (2014) in plants from SMF (2.9 mT–4.7 mT) treated wheat seeds. Alvarez et al. (2020) suggested that the adverse effect of salinity and drought on water absorption in *Triticale* seeds can be circumvented by exposing seeds to SMF of 3.71 mT for 10 h. At the plant level, soybean plants raised from SMF treated seeds and exposed to water stress at vegetative, flowering, and pod-filling stage showed lesser reduction in growth, photosynthetic efficiency, and yield (Baghel et al., 2018). Recently, the beneficial effects of magneto-priming (200 mT for 5 min) on improvement of drought tolerance in *Hyssopus officinalis* L. through protection of cellular membrane integrity, maintenance of photosynthetic pigment content, alternation of antioxidant enzyme activities were demonstrated by Mohammadi & Roshandel (2020).

#### **4.2.3 TEMPERATURE STRESS**

Among the various abiotic stresses experienced by plants, extreme temperatures have a detrimental effect on the metabolic activities of the plants. Seed

priming techniques are used by various researchers to combat temperature stress at later stages of growth in various crops (Thakur et al., 2019). Afzal et al. (2015) reported that maize treated with magnetic field of strength 150 mT increased chilling tolerance, plant growth, chlorophyll, total phenolics, gaseous exchange, seed protein, and oil content. In radish, magnetic field treatment (400 A/m) increased polar lipids under light and chilling stresses (Novitskaya et al., 2010). However, the effect of magneto-priming may generate interesting information if focused on the 'stress memory' response in relation to activation of gene network during priming and the mechanism of tolerance in plants on subsequent exposure to extreme temperatures.

#### **4.2.4 HEAVY METAL STRESS**

Magneto-priming may be used to ward off the deleterious effects of heavy metal stress by enhancing the seed germination, seedling vigor, rate of photosynthesis, biomass accumulation and crop productivity (Prajapati et al., 2020). Chen et al. (2011) reported that SMF (600 mT) alleviated the toxic effects of cadmium salts through increasing the photosynthetic rate, NO concentration and NOS activity and reducing the lipid peroxidation in mungbean seedlings. Pretreatment of SMF (200 mT for 1 h) to soybean ameliorated arsenic toxicity in relation to growth, photosynthesis, and water uptake (Fatima et al., 2020). In this study, SMF-pretreatment caused an increase in leaf area with larger thickness of midrib/minor veins along with improved photosynthetic performance in contrast to un-primed seeds under As(V) stress.

Iron uptake in plants is dependent on the redox reaction catalyzed by ferric reductase activity and the exposure to magnetic field alters the rates of redox reaction in plants (Berteau et al., 2015). Islam et al. (2020) suggested a role of the geomagnetic field in the uptake and maintenance of iron in the plant cells through increased activity of ferric reductase. These findings can be effectively utilized for phytoremediation of heavy metals like Fe through magneto-priming seeds before sowing in contaminated soils.

#### **4.2.5 UV-B STRESS**

Higher levels of biologically effective UV-B radiation traverse through the atmosphere due to high concentrations of ozone-depleting substances, changing climatic conditions, and altered land-use patterns (Laube et al., 2014; Bornman et al., 2015). The UV-B radiations have deleterious effect on important biomolecules (Kataria et al., 2014a; Parihar et al., 2015) and

adversely affect growth, leaf thickness, photosynthesis, nitrogen fixation and yield (Rozema et al., 2002; Turunen & Latola, 2005; Kataria et al., 2014a, b). The disruption of membrane systems due to increase in  $O_2^{\cdot-}$  radical along with increased malondialdehyde concentration and relative electrolyte conductivity in leaves of rice plants and cucumber cotyledons have been reported (Dai et al., 1997; Jain et al., 2004). On the other hand, lower levels of  $O_2^{\cdot-}$  radicals were found in leaves of maize plants that emerged after SMF-treatment (Shine & Guruprasad, 2012a, b).

The inhibitory effect of ambient UV stress in maize was alleviated by SMF-pre-treatment (Kataria et al., 2015). In a follow up study, it was seen that SMF (200 mT for 1 h) treatment of soybean seeds increased plant height, leaf area, biomass accumulation, hemichrome content in the root nodules, and improved the PS II efficiency and rate of photosynthesis both in presence and absence of solar UV in comparison to untreated seeds (Kataria et al., 2017b). Raipuria et al. (2021) observed that SMF-priming provides UV-B tolerance to soybean seedlings *via* increased ROS and NO content; and up-regulation of  $\alpha$ -amylase, nitric oxide NOS, and NR genes during the seed germination. Kataria et al. (2017b, 2020b, 2021) provided evidence that SMF-pretreatment increased the tolerance of soybean plants to ambient and supplemental UV-B radiation in the field conditions through the higher PSII efficiency, quantum yield of electron transport, performance indices, rate of photosynthesis and lower MDA, proline, ROS, and antioxidant (AsA,  $\alpha$ -Tocopherol) content which subsequently increase the yield of soybean plants. Kataria et al. (2020b) also found that SMF-priming of soybean seeds (200 mT for 1 h) enhanced the CA in the leaves and nitrogenase activity in root nodules under non-stress and ambient UV-B stress as compared to their unprimed control plants.

### 4.3 HYDROPRIMING FOR ABIOTIC STRESS TOLERANCE

Due to sessile nature, plants continuously experience various abiotic stresses such as salinity, extreme temperature, and drought at different developmental stages. Germination is an important stage that is highly responsive to vary under different environmental conditions and controlled by various molecular, biochemical, and physiochemical processes involved in embryogenesis (Bewley, 1997; Bewley et al., 2013; Lutts et al., 2016; Rajjou et al., 2012). Abiotic stresses are often interrelated and cause a series of adverse changes in morphological, physiological, molecular, and biochemical that disturb plant growth, development, and production (Hussain et al., 2018).

These alterations may result in membrane damage (Korkmaz et al., 2010), oxidative stress (Shafi et al., 2009), reactive oxygen species (ROS) production (Suzuki & Mittler, 2006), and reduced cellular respiration (Sugie et al., 2006). Hydropriming is a simple, cost-effective, and promising method to improve abiotic stress tolerance of plants by an increase in seed emergence and synchronized germination (Jisha et al., 2013). This can be achieved by stimulation of pre-germinative metabolic processes, metabolic repair, osmotic adjustments, DNA processing, early reverse mobilization, energy metabolism, and endosperm weakening (Chen & Arora, 2011; Pandita et al., 2007; Varier et al., 2010). Moreover, germination is improved by regulating DNA repair pathways, nucleic acid and protein synthesis, accumulation of sterols and phospholipids, and enzyme degradation such as catalase, etc. (Afzal et al., 2002; Paparella et al., 2015; Rajjou et al., 2012). Furthermore, other factors like induced cell division, fluidity of plasma membrane, cell elongation, and production of stress-responsive proteins play critical roles in proper seedling development (Ibrahim, 2016). Priming procedures are responsible for inducing stress defense mechanisms associated with abiotic stresses such as increased antioxidant scavenging enzymes, accumulation of HSP (heat shock protein), activation of membrane efflux, transcriptional activation of peroxiredoxin, and LEA proteins (Catusse et al., 2011; Li et al., 2005; Varier et al., 2010).

Hydro priming is a simple, safe, and economical technique used to produce seedlings with increased capacity of osmotic adjustment thus enhanced seed establishment and crop yield under environmental stress (Kaur et al., 2002). The seeds are hydrated by immersing in distilled water for a defined period determined by seed imbibitions at an appropriate temperature. The seeds must be dried properly under shade to their original weight (Jisha et al., 2013). However, germination is suspended due to low osmotic and matric potential (Basra et al., 2003) but hydropriming induces biochemical and physiological modifications in seeds before germination. Modifications like increased permeability and lower viscosity of protoplast attribute to increased nutrient and water uptake thus positive seedling growth and resistance to dehydration (Jisha et al., 2013; Yagmur & Kaydan, 2008). The predominant feature of hydropriming is the correlation between higher water uptake and increased seedling growth (Yagmur & Kaydan, 2008). This methodology is helpful under adverse environmental conditions as it improves seed hydration and water uptake efficiency under these constraints (McDonald, 2000). It is essential to maintain optimum temperature and humidity to avoid uncontrolled water uptake and radicle protrusion (Taylor

et al., 1998). The crucial technique of hydropriming is drum priming, which comprises a drum enclosed with seed coupled with a boiler creating vapors that condense and water droplets form in the drum (Warren & Bennett, 1997). Many studies have stated improved stand establishment, seed vigor, and yield under suboptimal or optimal conditions along with three to four-fold increases in germination as compared to non-primed seeds. Some studies explained improved seed germination by hydropriming in wheat under saline conditions (Afzal et al., 2007; Harris et al., 2001; Nawaz et al., 2016; Roy & Srivastava, 1999), chickpea (Harris et al., 1999; Kamithi et al., 2016; Kaur et al., 2002), lentil (Ghassemi et al., 2008), safflower (Ashrafi & Razmjoo, 2010; Bastia et al., 1999), mountain rye (Ansari & Zadeh, 2012), watermelon (Sung & Chiu, 1995), pearl millet (Kumar et al., 2002). Hydropriming was effective in enhancing vegetative and reproductive growth stages and seedling emergence in maize (Dezfuli et al., 2008; Mohammadi et al., 2008; Nagar et al., 1998). Several food crops coriander, wheat, *Allium porrum* and in desert plants like cacti (Dubrovsky, 1996) showed synchronized early germination after hydropriming. Similarly, improved seed germination was observed in onion (Caseiro et al., 2004) cauliflower (Jisha et al., 2013), and in mustard (Srivastava et al., 2010). Therefore, hydropriming provides improved seedling emergence seed germination, and productivity of field crops that can be explained by the rapid emergence of shoots and roots, and better tolerance under adverse conditions (Lee-Suskoon et al., 1998).

#### **4.3.1 SALT**

Salt stress is one of the leading problems and major growth-limiting factors in agriculture, with one-third of the world's land affected by salinity (Flowers & Colmer, 2008). Other factors such as nonsystemic irrigation, natural weathering, or intense agriculture also contribute to salt stress (Hasanuzzaman & Fotopoulos, 2019). The excess build-up of salts reduces water potential in plants, as consequence plants don't get access to water (Munns et al., 2006). Further reduction of water and nutrient uptake due to ion toxicity is observed (Chinnusamy et al., 2005). The impact of salinity includes, reduced water and nutrient uptake, altered rate of respiration, reduced rate of photosynthesis, lowered transpiration, ion toxicity, and affects stomatal conductance. When a plant is exposed to salinity, firstly the osmotic (hyperosmotic) stress happens due to excess sodium in the root zone, followed by ionic (hypertonic) stress due to altered concentration of essential nutrients (Munns et al., 2006). An increase in sodium levels reduces potassium ion influx and disturbs the

osmotic balance, enzymatic activities, and increase in ROS levels (Parida & Das, 2005; Parihar et al., 2015), altered stomatal functions, and nutrient imbalance in plants (Munns et al., 2006). Other physiological processes like seed germination, growth, flowering, and fruiting are also affected due to high levels of sodium (Parihar et al., 2015; Singh et al., 2013). However, plants may induce different morphological and physiological adaptations to acquire salt stress tolerance without affecting the normal functioning of the plant (Farooq et al., 2015; Shannon & Grieve, 1998). Under stress, the quality and composition of grain may change due to excess of a certain metabolite that helps the plant to tolerate the adverse effects of environmental factors. Once exposed to one stress, plants may exhibit tolerance to other or consequent stress in coming generations (Ćuk et al., 2010). Such transgenerational memory involves modifications in proteome and metabolome with upregulated expression of solutions and proteins required to overcome stress (Joyce et al., 2003; Walter et al., 2013). Seed priming induces metabolic alterations during seed germination and enhances osmolyte accumulation (Delavari et al., 2010), and lipid peroxidation, antioxidant activities, and proteins (Afzal et al., 2008; Jafar et al., 2012). The production of metabolites aids in setting up a defense mechanism. Seed priming has been efficient for salt tolerance in wheat (Jafar et al., 2012), mustard (Srivastava et al., 2010), maize (Aliu et al., 2015), and barley (Rashid et al., 2006). In *Solanum villosum*, the hydroprimed seeds showed synchronized germination, low oxidative stress, higher content of chlorogenic acid iso-orientin, and rutin. Hydropriming of maize seeds for 48 hrs. was beneficial in tolerating salinity stress. Similarly, hydroprimed sunflower seeds displayed better vigor and high germination rate under salinity stress (Matias et al., 2018).

#### **4.3.2 DROUGHT STRESS**

Water scarcity is considered to be one of the most destructive factors and a serious threat to agriculture, mostly in arid and semi-arid areas of the world. Inadequate rainfall, modern agriculture systems, and climate change are major causes of drought (Lobell et al., 2011). It adversely affects the germination, growth, and development of the plant. The severity of drought is unpredictable owing to the moisture content of soil, evaporation, and rainfall, etc. (Farooq et al., 2009). Drought can happen in all stages, and severely affects seed germination (Harris et al., 2002) and early seedling growth (Okçu et al., 2005), and crop stand (Kaya et al., 2006). In alfalfa, it was found that drought alters the fresh and dry weight of shoot and root, rate of

germination and hypocotyl length (Zeid & Shedeed, 2006), plant growth, and development in rice (Manickavelu et al., 2006). Drought stress reduced the spikes per plant, number of grains and number of tillers in barley (*Hordeum vulgare*) (Samarah, 2005), reduced the yield in pigeon pea by 40–55% (Nam et al., 2001) resulting in poor seed material. Seed priming induces drought resistance through changes in molecular mechanisms such as stress proteins and AQP, hormone signaling, cell membrane stability, osmotic adjustments, antioxidant defense, and morphological mechanisms by phenotypic plasticity. Thus, the use of different seed priming agents is encouraged (Farooq et al., 2009). Hydropriming was used for seed invigoration for effective germination of seeds in maize (Janmohammadi et al., 2008), seed growth in chickpea (Kaur et al., 2002), and cotton (Casenave & Toselli, 2007) under salinity and drought. Nadali et al. (2020) evaluated quinoa cultivars for drought stress response. Hydro priming significantly enhanced growth and seed yield along with a comparatively lower lipid peroxidation and electrolyte leakage in all the three quinoa cultivars. Hydropriming increased germination and seedling growth under salt and drought stresses in sunflower (Kaya et al., 2006).

### **4.3.3 HEAVY METAL STRESS**

Plants exhibit varied response to heavy metal phytotoxicity, that may occur due to volcanic eruptions, forest fires, surface mineralization, and spontaneous combustion or by human actions such as mining and agriculture (Nagajyoti et al., 2010). Human-produced contamination occurs through automobiles, use of pesticides, fertilizers, generator stations, metal smelters, mills, refineries, and municipal incinerators (El-Ramady et al., 2015). Heavy metals that require the growth and development of plants can be categorized as essential and nonessential nutrients. The essential nutrients are also known as micronutrients such as Cu, Ni, Zn, Mo, Fe, Co, and Mn, involved in different metabolic processes such as redox reaction and electron transfer, etc. The nonessential nutrients are toxic for plants such as Cd, Cr, As, Pb, and Hg (Rai et al., 2004; Sebastiani et al., 2004). Metals can further be classified based on physiochemical functions as redox and nonredox metals. The redox metals, i.e., Fe, Cu, Cr, and Mn (Jozefczak et al., 2012; Valko et al., 2005) cause oxidative injury that produces ROS resulting in DNA breakage, damaged photosynthesis mechanism, defragmented proteins, disruption in the cell and cell death (Flora, 2009; Schutzendubel, 2002). Non-redox metals include Hg, Al, Ni, cadmium, and Zn (Jozefczak et al., 2012; Valko et al., 2005) are involved in binding of sulfhydryl group, glutathione depletion



(Valko et al., 2005), and inhibition antioxidants that cause damage to plants (Bielen et al., 2013).

Heavy metals often reduce germination rates, reduce root, and shoot elongation rates, soluble protein content, and dry mass (Wang et al., 2003). Various heavy metals such as Cd, Hg, Ni, Pb, and Cr have toxic roles that affect productivity and affect germination (Ghosh & Sethy, 2013). In the case of  $\text{HgCl}_2$ , Hg reacts with the sulfhydryl groups and alters cell membrane permeability, therefore, inhibits germination (Bose et al., 1983a, b). Along with germination, heavy metals also show affinity towards phosphate groups, essential ions and causes oxidative stress. Plants have developed various sophisticated mechanisms to overcome heavy metal stress, this involves physical barriers such as thick cuticles, cell wall, mycorrhizal association, and trichome tissues that store heavy metals and detoxify by producing secondary metabolites (Hall, 2002; Harada et al., 2010; Hauser, 2014; Lee et al., 2002; Wong et al., 2004). Another mechanism used by plants is the production of proteins called chelators such as spermine, nicotianamine, phytochelatin, glutathione, organic acid, or cellular exudates such as phenolics, HSPs, phenolics, and flavonoids, and amino acids, i.e., proline and histamine, and hormones like jasmonic acid and salicylic acid, etc. (Dalvi & Bhalerao, 2013; Sharma & Dietz, 2006; Viehweger, 2014). However, hydropriming and halo priming by  $\text{Mg}(\text{NO}_3)_2$  and  $\text{Ca}(\text{NO}_3)_2$  improve germination percentage, seed emergence, radicle, and plumule length,  $\alpha$ -amylase activity, and soluble sugar content in endosperm in wheat (Kumar et al., 2016). Hydropriming enhanced the seed germination of *Medicago truncatula* in heavy metal contaminated soil. There was upregulation of genes involved in DNA damage repair and antioxidant defense in hydroprimed plant material (Forti et al., 2020).

#### **4.3.4 TEMPERATURE STRESS**

Temperature is considered to be one of the major factors which play a vital role in seed germination and various enzymatic and biochemical reactions required for the germination of seeds (Bewley et al., 2013; Nascimento et al., 2021) Temperature stress can be categorized into three types, i.e., chilling, freezing or high temperature. Temperature stress can develop by increase or decrease in temperature, duration, growth stage, and the rate at which temperature changes. Under stress, plant shows growth retardation, low germination rate and often death. However, plants exhibit various mechanisms to overcome temperature stress, i.e., molecular mechanism

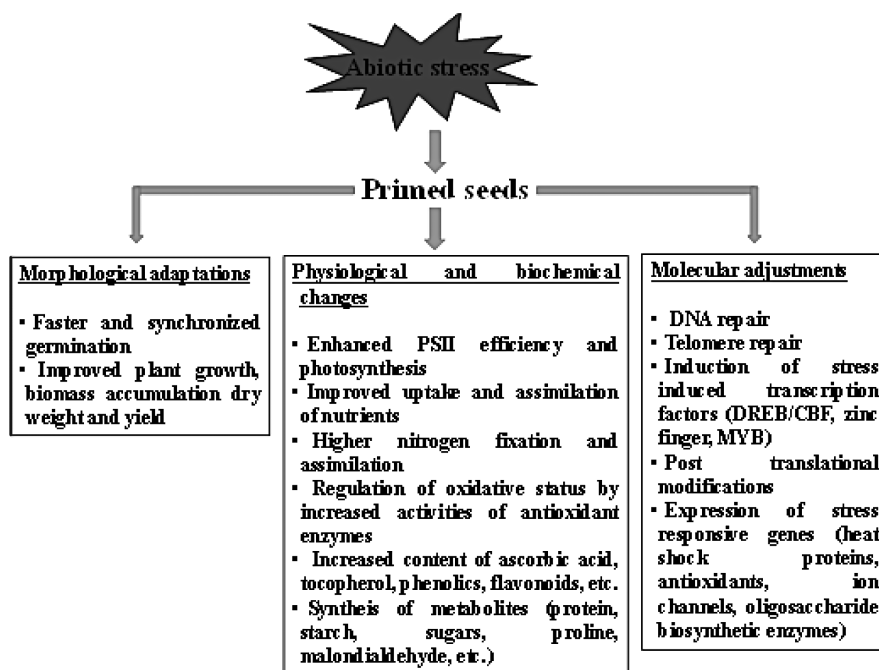
with metabolites, proteins, antioxidants, membrane lipids, stress-related transcription factors, regulatory factors, etc. (Kai & Iba, 2014).

Temperature raised above ambient temperature by 10–15°C results in heat shock and harms the growth, development, and production of plants (Hall, 2001). This involves, denaturation of proteins, inhibition of enzymes, alteration of membrane integrity (Howarth, 2005), physiological process, root, and shoot inhibition, moisture content, seed, and tiller size (Iqbal et al., 2017), reduced photosynthesis and respiration (Barnabás et al., 2007), abscission and senescence (Hasanuzzaman et al., 2013; Vollenweider & Günthardt-Goerg, 2005). It also alters the gene expression levels, accumulation of transcripts and initiates stress response by synthesis of genes involved in stress response (Iba, 2002). Low temperature or chilling stress also has harmful effects by interfering in the normal functioning of plants systems. However, plants induce different mechanisms to overcome stress by making short- and long-term alterations in molecular, and morphological mechanisms, i.e., leaf orientation, membrane composition, transpiration, etc. (Adam et al., 2011). Pre-treatment of seeds can be beneficial to increase tolerance against thermal stress (Nascimento et al., 2021). This is done by seed priming that promotes protein synthesis for HSPs, and enzymes required for tissue repair such as L-isoaspartyl, methyltransferase, etc., to increase low and heat temperature stress tolerance (Ji et al., 2017; Kester et al., 1997). Various studies have reported germination under cold stress with improved crop yield in hydroprimed maize (Finch-Savage et al., 2004) and resulted in faster germination of chickpea (Elkoca et al., 2007). Hydropriming could alleviate the negative impact of cold temperature on the plant performance in Mungbean and narrow-leaf lupine (*Lupine angustifolius*) (Płazek et al., 2018; Posmyk & Janas, 2007). It was shown that resistance to chilling can be improved better seed hydration (Knypl, 1979; Posmyk & Janas, 2007), possibly due to changes in membrane conformation (Chen & Arora, 2013).

#### 4.4 CONCLUSIONS

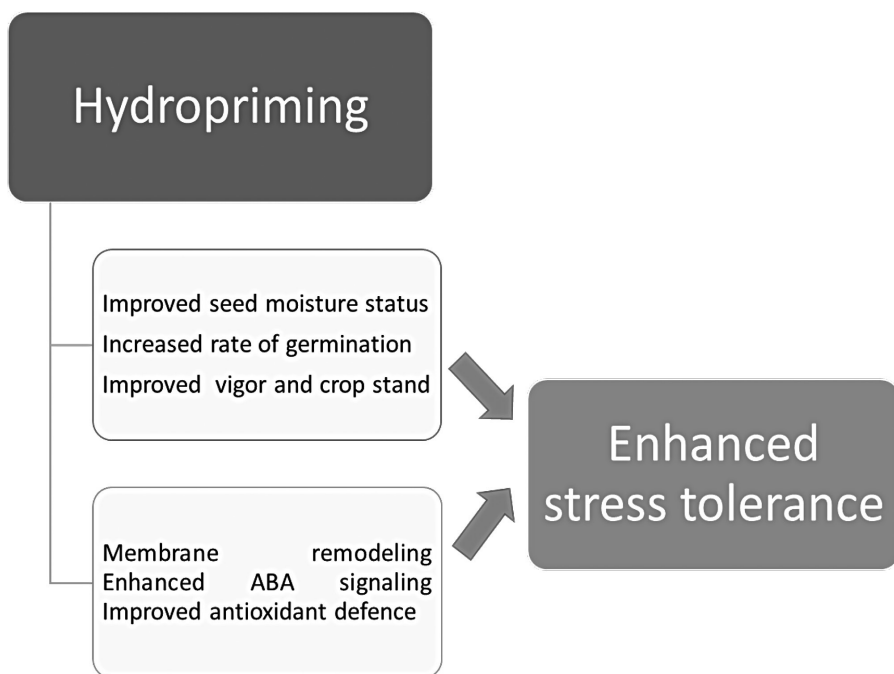
Uncertain and adverse environmental conditions during sowing compel the farmers to use high seed rate to compensate for poor emergence and achieve a normal crop stand. Seed priming is a well-recognized seed enhancement technique to enhance the planting value of various crops as it improves the seed performance under suboptimal sowing conditions. It induces morphological adaptations accompanied by physiological, biochemical, and molecular changes in plant cells under environmental stresses (Figure 4.1). Morphological

adaptations through seed-priming include hastening and synchronization of germination; improvement in plant growth, plant height, leaf area, root-shoot length, photosynthesis, accumulation of biomass to minimize or undo the effect on yield (Prajapati et al., 2020). During priming, hastened metabolic activity converts stored reserves into simpler compounds necessary for faster germination rates (Gallardo et al., 2001). It triggers the cell cycle-related processes and increases activities of antioxidant enzymes including superoxide dismutase, catalase, peroxidase, glutathione reductase (GR), etc., that ultimately improve oxidative status of plants under stress conditions, leading to increased seed germination and vigor (Khan et al., 2020). Seed priming is also known to improve seed viability by inducing the DNA repair machinery for repair of damaged DNA formed during the aging process (Thornton et al., 1993). The study reported that pre-replicative repair of damaged DNA is carried out during the aerated hydration that takes place during the priming treatments. Priming enhances the protein synthesis by improving the proper functioning of protein-synthesizing machinery like enhanced rRNA synthesis and maintenance of ribosome integrity (Coolbear et al., 1990).



**FIGURE 4.1** Seed priming induced morphological, physiological, biochemical, and molecular changes in plants under various abiotic stresses.

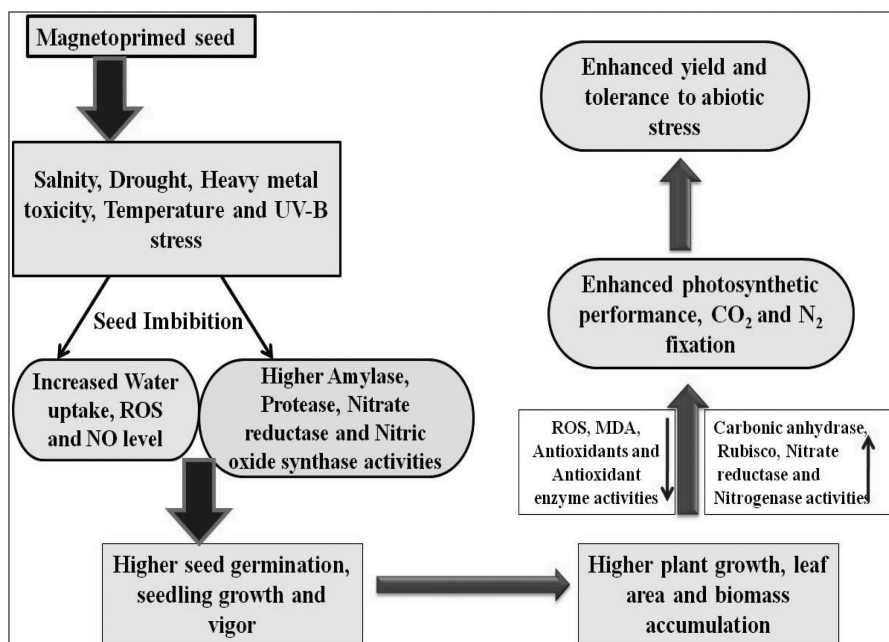
There was upregulation of genes involved in DNA damage repair and anti-oxidant defense in hydro primed plant material (Figure 4.2). Hydropriming allows enhances seed moisture status moisture, shortens imbibition duration along with constant supply of oxygen, thus increasing the level of metabolites associated with the germination process. Rather, hydropriming is considered as an early germination process at an optimal priming environment. In *Arabidopsis*, a hydropriming-specific Catalase isoform is found (Gallardo et al., 2001). In sugar beet seeds, hydropriming induces the accumulation of ABA signaling intermediates protein phosphatase 2C and 14-3-3 proteins (Catusse et al., 2011).



**FIGURE 4.2** Effect of hydropriming on the seed germination and plant growth under abiotic stresses.

The morpho-physiological and biochemical responses of magnetoprimed seeds to various stresses are summarized in Figure 4.3. Figure 4.3 illustrates that magneto-priming enhances the seed germination and seedling growth due to increase in the level of ROS, NO which may have a signaling role. The increased activities of amylase and protease in the germinating seeds can lead to a faster rate of germination. This stimulatory effect of magneto-priming

persists till its maturity as evidenced by stimulated plant growth, leaf area, biomass, and improved the nitrogen fixation, photosynthetic performance and CO<sub>2</sub> fixation which results in better yields under non-stress and abiotic stressed conditions.



**FIGURE 4.3** Effect of magneto-priming on the seed germination and plant growth under abiotic stresses.

## KEYWORDS

- glutathione reductase
- hydropriming
- late embryogenesis abundant protein
- magneto-priming
- stress tolerance
- superoxide dismutase

## REFERENCES

- Abbate, P. E., Dardanelli, J. L., Cantarero, M. G., Maturano, M., Melchiori, R. J. M., & Suero, E. E., (2004). Climatic and water availability effects on water-use efficiency in wheat. *Crop Sci.*, 44, 474–483. <https://doi.org/10.2135/cropsci2004.4740>.
- Adam, S., Cockshull, K., & Cave, C., (2011). Effect of temperature on the growth and development of tomato fruits. *Ann. Bot.*, 88, 869–877.
- Afzal, I., Basra, S. M. A., Ahmad, N., & Lodhi, T., (2007). Counteraction of salinity stress on wheat plants by pre-sowing seed treatments. *Pak. J. Agric. Sci.*, 104–109.
- Afzal, I., Basra, S. M. A., Ahmad, N., Cheema, M. A., Warraich, E. A., & Khaliq, A., (2002). Effect of priming and growth regulator treatment on emergence and seedling growth of hybrid maize (*Zea mays*). *Int. J. Agric. Biol.*, 4, 303–306.
- Afzal, I., Noor, M. A., Bakhtavar, M. A., Ahmad, A., & Haq, Z., (2015). Improvement of spring maize (*Zea mays*) performance through physical and physiological seed enhancements. *Seed Sci. Technol.*, 43, 1–12.
- Afzal, I., Rauf, S., Basra, S. M. A., & Murtaza, G., (2008). Halopriming improves vigor, metabolism of reserves and ionic contents in wheat seedlings under salt stress. *Plant Soil Environ.*, 54, 382–388.
- Ahmad, S., Ahmad, R., Ashraf, M. Y., Ashraf, M., & Waraich, E. A., (2009). Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pak J. Bot.*, 41, 647–654.
- Aliu, S., Rusinovci, I., Fetahu, S., Gashi, B., Simeonovska, E., & Rozman, L., (2015). The effect of salt stress on the germination of maize (*Zea mays* L.) seeds and photosynthetic pigments. *AAS*, 105, 85–94. <https://doi.org/10.14720/aas.2015.105.1.09>.
- Alvarez, J., Martinez, E., Carbonell, V., & Florez, M., (2020). Effects of polyethylene glycol and sodium chloride stress on water absorption of magneto-primed triticale seeds. *Romanian Reports in Physics*, 72, 708.
- Anand, A., Kumari, A., Thakur, M., & Koul, A., (2019). Hydrogen peroxide signaling integrates with phytohormones during the germination of magneto primed tomato seeds. *Sci. Rep.*, 9, 8814. <https://doi.org/10.1038/s41598-019-45102-5>.
- Anand, A., Nagarajan, S., Verma, A. P. S., Joshi, D. K., Pathak, P. C., & Bhardwaj, J., (2012). Pre-treatment of seeds with static magnetic field ameliorates soil water stress in seedlings of maize (*Zea mays* L.). *Indian Journal of Biochemistry & Biophysics*, 49, 63–70.
- Anderson, A. J., McLean, J. E., Jacobson, A. R., & Britt, D. W., (2018). CuO and ZnO nanoparticles modify interkingdom cell signaling processes relevant to crop production. *J. Agric. Food Chem.*, 66, 6513–6524. <https://doi.org/10.1021/acs.jafc.7b01302>.
- Ansari, O., & Zadeh, F., (2012). Osmo and hydro priming improvement germination characteristics and enzyme activity of mountain rye (*Secale montanum*) seeds under drought stress. *J. Stress Physiol. Biochem.*, 8, 253–261.
- Araújo, S. S., Paparella, S., Dondi, D., Bentivoglio, A., Carbonera, D., & Balestrazzi, A., (2016). Physical methods for seed invigoration: Advantages and challenges in seed technology. *Frontiers in Plant Science*, 7, 646. <https://doi.org/10.3389/fpls.2016.00646>.
- Ashrafi, E., & Razmjoo, K., (2010). Effects of priming on seed germination and field emergence of safflower (*Carthamus tinctorius* L.). *Seed Sci. Technol.*, 38, 675–681. <https://doi.org/10.15258/sst.2010.38.3.15>.

- Baghel, L., Kataria, S., & Guruprasad, K. N., (2016). Static magnetic field treatment of seeds improves carbon and nitrogen metabolism under salinity stress in soybean. *Bioelectromagnetics*, 37, 455–470. <https://doi.org/10.1002/bem.21988>.
- Baghel, L., Kataria, S., & Guruprasad, K. N., (2018). Effect of static magnetic field pretreatment on growth, photosynthetic performance and yield of soybean under water stress. *Photosynthetica*, 56, 718–730. <https://doi.org/10.1007/s11099-017-0722-3>.
- Baghel, L., Kataria, S., & Jain, M., (2019). Mitigation of adverse effects of salt stress on germination, growth, photosynthetic efficiency and yield in maize (*Zea mays* L.) through magnetopriming. *Acta Agrobot.*, 72(1), 175. <https://doi.org/10.5586/aa.1757>.
- Bailly, C., Benamar, A., Corbineau, F., & Come, D., (2000). Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. *Seed Science Research*, 10, 35–42. <https://doi.org/10.1017/S0960258500000040>.
- Barnabás, B., Jäger, K., & Fehér, A., (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.*, 31(1), 11–38. <https://doi.org/10.1111/j.1365-3040.2007.01727.x>.
- Basra, S. M. A., Pannu, I., & Afzal, I., (2003). Evaluation of seedling vigor of hydro and matriprimed wheat (*Triticum aestivum* L.) seeds. *Int. J. Agric. Biol.*, 5, 121–123.
- Bastia, D., Rout, A., Mohanty, S., & Prusty, A., (1999). Effect of sowing date sowing methods and seed soaking on yield and oil content of rainfed safflower grown in Kalahandi, Orissa. *Indian J. Agron.*, 44, 621–623.
- Bertea, C. M., Narayana, R., Agliassa, C., Rodgers, C. T., & Maffei, M. E., (2015). Geomagnetic field (GMF) and plant evolution: Investigating the effects of GMF reversal on *Arabidopsis thaliana* development and gene expression. *J. Vis. Exp.*, 105, 53286. doi: 10.3791/53286.
- Bewley, J. D., (1997). Seed germination and dormancy. *Plant Cell*, 1055–1066. <https://doi.org/10.1105/tpc.9.7.1055>.
- Bewley, J. D., Bradford, K. J., Hillhorst, H., & Nonagaki, H., (2013). *Seeds: Physiology of Development, Germination and Dormancy*. Springer, New York.
- Bhardwaj, J., Anand, A., & Nagarajan, S., (2012). Biochemical and biophysical changes associated with magneto priming in germinating cucumber seeds. *Plant Physiol. Biochem.*, 57, 67–73. <https://doi.org/10.1016/j.plaphy.2012.05.008>.
- Bhardwaj, J., Anand, A., Pandita, V. K., & Nagarajan, S., (2016). Pulsed magnetic field improves seed quality of aged green pea seeds by homeostasis of free radical content. *J. Food Sci. Technol.*, 53(11), 3969–3977. <https://doi.org/10.1007/s13197-016-2392-8>.
- Bielen, A., Remans, T., Vangronsveld, J., & Cuypers, A., (2013). The influence of metal stress on the availability and redox state of ascorbate, and possible interference with its cellular functions. *IJMS*, 14, 6382–6413. <https://doi.org/10.3390/ijms14036382>.
- Blokhina, O., (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany*, 91, 179–194. <https://doi.org/10.1093/aob/mcf118>.
- Bornman, J. F., Barnes, P. W., Robinson, S. A., Ballaré, C. L., Flint, S. D., & Caldwell, M. M., (2015). Solar ultraviolet radiation and ozone depletion-driven climate change: Effects on terrestrial ecosystems. *Photochem. Photobiol. Sci.*, 14, 88–107.
- Bose, B., Srivastava, H., & Mathur, S., (1983a). Partial purification and properties of protease from maize seed endosperm. *Beitrag Biologie der Pflanzen*, 58, 383–391.
- Bose, B., Srivastava, R., Singh, V., & Mathur, S., (1983b). Sodium hydrogen carbonate: A potent electron donor for the nitrogen utilizing enzymes in *Vigna mungo*. *Biochem. Physiol. Pflanz.*, 178, 443–448.

- Caseiro, R., Bennett, M. A., & Marcos-Filho, J., (2004). Comparison of three priming techniques for onion seed lots differing in initial seed quality. *Seed Sci. Technol.*, 32, 365–375. <https://doi.org/10.15258/sst.2004.32.2.09>.
- Casenave, E. C., & Toselli, M. E., (2007). Hydropriming as a pre-treatment for cotton germination under thermal and water stress conditions. *Seed Sci. Technol.*, 35, 88–98. <https://doi.org/10.15258/sst.2007.35.1.08>.
- Catusse, J., Meinhard, J., Job, C., Strub, J. M., Fischer, U., Pestsova, E., et al., (2011). Proteomics reveals potential biomarkers of seed vigor in sugar beet. *Proteomics*, 11(9), 1569–1580. <https://doi.org/10.1002/PMIC.201000586>.
- Chen, K., & Arora, R., (2011). Dynamics of the antioxidant system during seed osmopriming, post-priming germination, and seedling establishment in Spinach (*Spinacia oleracea*). *Plant Science*, 180, 212–220. <https://doi.org/10.1016/j.plantsci.2010.08.007>.
- Chen, K., & Arora, R., (2013). Priming memory invokes seed stress-tolerance. *Environmental and Experimental Botany*, 94, 33–45. <https://doi.org/10.1016/j.envexpbot.2012.03.005>.
- Chen, K., Fessehaie, A., & Arora, R., (2013). Aquaporin expression during seed osmopriming and post-priming germination in spinach. *Biologia Plant.*, 57, 193–198. <https://doi.org/10.1007/s10535-012-0266-0>.
- Chen, Y. P., Li, R., & He, J. M., (2011). Magnetic field can alleviate toxicological effect induced by cadmium in mungbean seedlings. *Ecotoxicology*, 20, 760–769. <https://doi.org/10.1007/s10646-011-0620-6>.
- Chinnusamy, V., Jagendorf, A., & Zhu, J. K., (2005). Understanding and improving salt tolerance in plants. *Crop Sci.*, 45, 437–448.
- Coolbear, P., Slater, R. J., & Bryant, A., (1990). Changes in nucleic acid levels associated with improved germination performance of tomato seeds after low-temperature presowing treatment. *Annals of Botany*, 65, 187–195. <https://doi.org/10.1093/oxfordjournals.aob.a087923>.
- Ćuk, M., Gladman, B. J., & Stewart, S. T., (2010). Constraints on the source of lunar cataclysm impactors. *Icarus*, 207, 590–594. <https://doi.org/10.1016/j.icarus.2009.12.013>.
- Dai, Q., Yan, B., Huang, S., Liu, X., Peng, S., Miranda, M. L., Chavez, A. Q., et al., (1997). Response of oxidative stress defense systems in rice (*Oryza sativa*) leaves with supplemental UV-B radiation. *Physiol. Plant.*, 101, 301–308.
- Dalvi, A., & Bhalerao, S., (2013). Response of plants towards heavy metal toxicity: An overview of avoidance, tolerance and uptake mechanism. *Ann. Plant Sci.*, 2, 362–368.
- De Souza, A., Garcia, D., Sueiro, L., Gilart, F., Porras, E., & Licea, L., (2006). Pre-sowing magnetic treatments of tomato seeds increase the growth and yield of plants. *Bioelectromagnetics*, 27, 247–257.
- Delavari, P. M., Baghizadeh, A., Enteshari, S. H., Kalantari, Kh. M., Yazdanpanah, A., & Mousavi, E. A., (2010). The effects of salicylic acid on some of the biochemical and morphological characteristic of *Ocimum basilicum* under salinity stress. *Australian Journal of Basic and Applied Sciences*, 4, 4832–4845.
- Dezfuli, P., Zadeh, F., & Janmohammadi, M., (2008). Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). *J. Agric. Biol. Sci.*, 3, 22–25.
- Dubrovsky, J., (1996). Seed hydration memory in Sonoran Desert cacti and its ecological implication. *Am. J. Bot.*, 83, 624–632.
- Engilla, J. N., Davies, F. T., & Boutton, T. W., (2005). Drought stress influences leaf water content, photosynthesis, and water-use efficiency of hibiscus *rosa-sinensis* at three potassium concentrations. *Photosynth.*, 43, 135–140. <https://doi.org/10.1007/s11099-005-5140-2>.



- Elkoca, E., Haliloglu, K., Esitken, A., & Ercisli, S., (2007). Hydro- and osmopriming improve chickpea germination. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science*, 57, 193–200. <https://doi.org/10.1080/09064710600914087>.
- El-Ramady, H., Abdalla, N., Alshaal, T., Domokos-Szabolcsy, É., Elhawaw, N., Prokisch, J., Sztrik, A., et al., (2015). Selenium in soils under climate change, implication for human health. *Environ. Chem. Lett.*, 13, 1–19. <https://doi.org/10.1007/s10311-014-0480-4>.
- El-Yazied, A., Shalaby, O., El-Gizawy, A., Khalf, S., & El-Satar, A., (2011). Effect of magnetic field on seed germination and transplant growth of tomato. *J. Am. Sci.*, 7, 306–312.
- Farooq, M., Barsa, S. M. A., & Wahid, A., (2006a). Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. *Plant Growth Regul.*, 49, 285–294. <https://doi.org/10.1007/s10725-006-9138-y>.
- Farooq, M., Basra, S. M. A., & Ahmad, N., (2007). Improving the performance of transplanted rice by seed priming. *Plant Growth Regul.*, 51, 129–137. <https://doi.org/10.1007/s10725-006-9155-x>.
- Farooq, M., Basra, S. M. A., & Asad, S. A., (2008). Comparison of conventional puddling and dry tillage in rice-wheat system. *Paddy Water Environ.*, 6, 397–404. <https://doi.org/10.1007/s10333-008-0138-6>.
- Farooq, M., Basra, S. M. A., Afzal, I., & Khaliq, A., (2006a). Optimization of hydropriming techniques for rice seed invigoration. *Seed Science and Technology*, 34, 507–512.
- Farooq, M., Basra, S. M. A., Ahmad, N., & Hafeez, K., (2005). Thermal hardening: A new seed vigor enhancement tool in rice. *J. Integrative Plant Biology*, 47, 187–193. <https://doi.org/10.1111/j.1744-7909.2005.00031.x>.
- Farooq, M., Basra, S. M. A., Khalid, M., Tabassum, R., & Mahmood, T., (2006b). Nutrient homeostasis, metabolism of reserves, and seedling vigor as affected by seed priming in coarse rice. *Can. J. Bot.*, 84, 1196–1202. <https://doi.org/10.1139/b06-088>.
- Farooq, M., Basra, S. M. A., Rehman, H., & Saleem, B. A., (2008). Seed priming enhances the performance of late sown wheat (*Triticum aestivum* L.) by improving chilling tolerance. *J. Agron. Crop Sci.*, 194, 55–60. <https://doi.org/10.1111/j.1439-037X.2007.00287.x>.
- Farooq, M., Basra, S. M. A., Tabassum, R., & Afzal, I., (2006b). Enhancing the performance of direct seeded fine rice by seed priming. *Plant Production Science*, 9, 446–456. <https://doi.org/10.1626/pps.9.446>.
- Farooq, M., Basra, S. M., Wahid, A., & Ahmad, N., (2010). Changes in nutrient-homeostasis and reserves metabolism during rice seed priming: Consequences for seedling emergence and growth. *Agricultural Sciences in China*, 9, 191–198. [https://doi.org/10.1016/S1671-2927\(09\)60083-3](https://doi.org/10.1016/S1671-2927(09)60083-3).
- Farooq, M., Hussain, M., Wakeel, A., & Siddique, K. H. M., (2015). Salt stress in maize: Effects, resistance mechanisms, and management. A review. *Agron. Sustain. Dev.*, 35, 461–481. <https://doi.org/10.1007/s13593-015-0287-0>.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S., (2009). Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev.*, 29, 185–212.
- Fashui, H., (2002). Study on the mechanism of cerium nitrate effects on germination of aged rice seed. *BTER*, 87, 191–200. <https://doi.org/10.1385/BTER:87:1-3:191>.
- Fatima, A., Kataria, S., Prajapati, S., Jain, M., Agrawal, A. K., Singh, B., Kashyap, Y., et al., (2020). Magnetopriming effects on arsenic stress-induced morphological and physiological variations in soybean involving synchrotron imaging. *Physiol. Plant*. <https://doi.org/10.1111/ppl.13211>.

- Finch-Savage, W. E., Dent, K. C., & Clark, L. J., (2004). Soak conditions and temperature following sowing influence the response of maize (*Zea mays* L.) seeds to on-farm priming (pre-sowing seed soak). *Field Crops Research*, 90, 361–374. <https://doi.org/10.1016/j.fcr.2004.04.006>.
- Flora, S. J. S., (2009). Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxid Med Cell Longev.*, 2, 191–206. <https://doi.org/10.4161/oxim.2.4.9112>.
- Florez, M., Carbonell, M. V., & Martínez, E., (2004). Early sprouting and first stages of growth of rice seeds exposed to a magnetic field. *Electro-Magnetobiol.*, 23, 167–176.
- Flowers, T. J., & Colmer, T. D., (2008). Salinity tolerance in halophytes. *New Phytologist*, 179, 945–963. <https://doi.org/10.1111/j.1469-8137.2008.02531.x>.
- Forti, C., Shankar, A., Singh, A., Balestrazzi, A., Prasad, V., & Macovei, A., (2020). Hydropriming and bioprimering improve *Medicago truncatula* seed germination and upregulate DNA repair and antioxidant genes. *Genes*, 11(3), 242. <https://doi.org/10.3390/GENES11030242>.
- Fu, J., & Huang, B., (2001). Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany*, 45, 105–114. [https://doi.org/10.1016/S0098-8472\(00\)00084-8](https://doi.org/10.1016/S0098-8472(00)00084-8).
- Gallardo, K., Job, C., Groot, S. P. C., Puype, M., Demol, H., Vandekerckhove, J., & Job, D., (2001). Proteomic analysis of *Arabidopsis* seed germination and priming. *Plant Physiology*, 126(2), 835–848. <https://doi.org/10.1104/PP.126.2.835>.
- Garg, B., (2003). Nutrient uptake and management under drought: Nutrient-moisture interaction. *Curr Agric.*, 27, 1–8.
- Ghassemi, G., Aliloo, A., Valizadeh, M., & Moghaddam, M., (2008). Effect of hydro and osmo-priming on seed germination and field emergence of lentil (*Lens culinaris* Medik.). *Not. Bot. Horti. Agrobot Cluj.*, 361, 29–33.
- Ghosh, S., & Sethy, S., (2013). Effect of heavy metals on germination of seeds. *J. Nat. Sc. Biol. Med.*, 4, 272. <https://doi.org/10.4103/0976-9668.116964>.
- Gill, S. S., & Tuteja, N., (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12), 909–930.
- Gupta, M. K., Anand, A., Paul, V., Dahuja, A., & Singh, A. K., (2015). Reactive oxygen species-mediated improvement in vigor of static and pulsed magneto-primed cherry tomato seeds. *Ind. J. Plant Physiol.*, 20(3), 205–212.
- Gust, A. A., Brunner, F., & Nürnberger, T., (2010). Biotechnological concepts for improving plant innate immunity. *Current Opinion in Biotechnology*, 21, 204–210. <https://doi.org/10.1016/j.copbio.2010.02.004>.
- Hall, A., (2001). Crop developmental responses to temperature, photoperiod, and light quality. In: *Crop Response to Environment* (pp. 83–87).
- Hall, J. L., (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.*, 53, 1–11.
- Harada, E., Kim, J. A., Meyer, A. J., Hell, R., Clemens, S., & Choi, Y. E., (2010). Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. *Plant Cell Physiol.*, 51, 1627–1637. <https://doi.org/10.1093/pcp/pcq118>.
- Hare, P. D., Cress, W. A., & Van, S. J., (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant Cell & Environment*, 21, 535–553. <https://doi.org/10.1046/j.1365-3040.1998.00309.x>.

- Harris, D., Joshi, A., Khan, P. A., Gothkar, P., & Sodhi, P. S., (1999). On-farm seed priming in semi-arid agriculture: Development and evaluation in maize, rice and chickpea in India using participatory methods. *Ex. Agric.*, 35, 15–29. <https://doi.org/10.1017/S0014479799001027>.
- Harris, D., Raghuwanshi, B. S., Gangwar, J. S., Singh, S. C., Joshi, K. D., Rashid, A., & Hollington, P. A., (2001). participatory evaluation by farmers of on-farm seed priming in wheat in India, Nepal and Pakistan. *Ex. Agric.*, 37, 403–415. <https://doi.org/10.1017/S0014479701003106>.
- Harris, D., Tripathi, R., & Joshi, A., (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. In: *Direct Seeding: Research Strategies and Opportunities* (pp. 231–240). IRRI, International Research Institute, Manila.
- Hasanuzzaman, M., & Fotopoulos, V., (2019). *Priming and Pretreatment of Seeds and Seedlings: Implication in Plant Stress Tolerance and Enhancing Productivity in Crop Plants*. Springer Singapore, Singapore. <https://doi.org/10.1007/978-981-13-8625-1>.
- Hasanuzzaman, M., Nahar, K., Fujita, M., Ahmad, P., Chandna, R., Prasad, M. N. V., & Ozturk, M., (2013). Enhancing plant productivity under salt stress: Relevance of poly-omics. In: Ahmad, P., Azooz, M. M., & Prasad, M. N. V., (eds.), *Salt Stress in Plants* (pp. 113–156). Springer New York, New York, NY. [https://doi.org/10.1007/978-1-4614-6108-1\\_6](https://doi.org/10.1007/978-1-4614-6108-1_6).
- Hasegawa, P. M., Bressan, R. A., Zhu, J. K., & Bohnert, H. J., (2000). Plant cellular and molecular responses to high salinity. *Annual Review of Plant Biology*, 51, 463–499. <https://doi.org/10.1146/annurev.arplant.51.1.463>.
- Hauser, M. T., (2014). Molecular basis of natural variation and environmental control of trichome patterning. *Front. Plant Sci.*, 5. <https://doi.org/10.3389/fpls.2014.00320>.
- Hill, H., Bradford, K. J., Cunningham, J., & Taylor, A. G., (2008). Primed lettuce seeds exhibit increased sensitivity to moisture during aging. *Acta Hort.*, 135–142. <https://doi.org/10.17660/ActaHortic.2008.782.14>.
- Howarth, C., (2005). Genetic improvements of tolerance to high temperature. In: *Abiotic Stresses—Plant Resistance Through Breeding and Molecular Approaches* (pp. 277–300). The Haworth Press, New York.
- Hozayn, M., EL-Mahdy, A. A., & Zalama, M. T., (2018). Magneto-priming for improving germination, seedling attributes and field performance of barley (*Hordeum vulgare* L.) under salinity stress. *Middle East Journal of Agriculture Research*, 7(3), 1006–1022.
- Huang, H. H., & Wang, S. R., (2008). The effects of inverter magnetic fields on early seed germination of mung beans. *Bioelectromagnetics*, 29, 649–657.
- Hussain, H. A., Hussain, S., Khaliq, A., Ashraf, U., Anjum, S. A., Men, S., & Wang, L., (2018). Chilling and drought stresses in crop plants: Implications, cross talk, and potential management opportunities. *Front. Plant Sci.*, 9, 393. <https://doi.org/10.3389/fpls.2018.00393>.
- Hussain, S., Khan, F., Hussain, H. A., & Nie, L., (2016). Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. *Front. Plant Sci.*, 7. <https://doi.org/10.3389/fpls.2016.00116>.
- Hussain, S., Zheng, M., Khan, F., Khaliq, A., Fahad, S., Peng, S., Huang, J., et al., (2015). Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. *Sci. Rep.*, 5, 8101. <https://doi.org/10.1038/srep08101>.
- Iba, K., (2002). Acclimative response to temperature stress in higher plants: Approaches of gene engineering for temperature tolerance. *Annu. Rev. Plant Biol.*, 53, 225–245. <https://doi.org/10.1146/annurev.arplant.53.100201.160729>.

- Ibrahim, E. A., (2016). Seed priming to alleviate salinity stress in germinating seeds. *Journal of Plant Physiology*, 192, 38–46. <https://doi.org/10.1016/j.jplph.2015.12.011>.
- Iqbal, M., & Ashraf, M., (2010). Changes in hormonal balance: A possible mechanism of presowing chilling-induced salt tolerance in spring wheat. *Journal of Agronomy & Crop Science*, 196, 440–454. <https://doi.org/10.1111/j.1439-037X.2010.00434.x>.
- Iqbal, M., Raja, N. I., Yasmeen, F., Hussain, M., Ejaz, M., & Shah, M. A., (2017). Impacts of heat stress on wheat: A critical review. *Adv. Crop Sci. Tech.*, 5. <https://doi.org/10.4172/2329-8863.1000251>.
- Islam, M., Maffei, M. E., & Vigani, G., (2020). The geomagnetic field is a contributing factor for an efficient iron uptake in *Arabidopsis thaliana*. *Front. Plant Sci.*, 11, 325. doi: 10.3389/fpls.2020.00325.
- Jafar, M. Z., Farooq, M., Cheema, M. A., Afzal, I., Basra, S. M. A., Wahid, M. A., Aziz, T., & Shahid, M., (2012). Improving the performance of wheat by seed priming under saline conditions: Salinity tolerance in wheat. *Journal of Agronomy and Crop Science*, 198, 38–45. <https://doi.org/10.1111/j.1439-037X.2011.00485.x>.
- Jain, K., Kataria, S., & Guruprasad, K. N., (2003). Changes in antioxidant defenses of cucumber cotyledons in response to UV-B and to the free radical generating compound AAPH. *Plant Science*, 165, 551–557.
- Janmohammadi, M., Moradi, D. P., & Sharifzadeh, F., (2008). Seed invigoration techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. *Gen. Appl. Plant Physiol.*, 34, 215–226.
- Javed, N., Ashraf, M., Akram, N. A., & Al-Qurainy, F., (2011). Alleviation of adverse effects of drought stress on growth and some potential physiological attributes in maize (*Zea mays* L.) by seed electromagnetic treatment. *Photochem. Photobiol.*, 87, 1354–1362.
- Ji, C. Y., Jin, R., Xu, Z., Kim, H. S., Lee, C. J., Kang, L., Kim, S. E., et al., (2017). Overexpression of *Arabidopsis* P3B increases heat and low temperature stress tolerance in transgenic sweetpotato. *BMC Plant Biol.*, 17, 139. <https://doi.org/10.1186/s12870-017-1087-2>.
- Jin, Y., Guo, W., Hu, X., et al., (2019). Static magnetic field regulates *Arabidopsis* root growth via auxin signaling. *Sci. Rep.*, 9, 14384. <https://doi.org/10.1038/s41598-019-50970-y>.
- Jisha, K. C., Vijayakumari, K., & Puthur, J. T., (2013). Seed priming for abiotic stress tolerance: An overview. *Acta Physiol. Plant.*, 35, 1381–1396. <https://doi.org/10.1007/s11738-012-1186-5>.
- Joyce, P. R., McKenzie, J. M., Luty, S. E., Mulder, R. T., Carter, J. D., Sullivan, P. F., & Robert, C. C., (2003). Temperament, childhood environment and psychopathology as risk factors for avoidant and borderline personality disorders. *Aust N Z J. Psychiatry*, 37, 756–764. <https://doi.org/10.1080/j.1440-1614.2003.01263.x>.
- Jozefczak, M., Remans, T., Vangronsveld, J., & Cuypers, A., (2012). Glutathione is a key player in metal-induced oxidative stress defenses. *IJMS*, 13, 3145–3175. <https://doi.org/10.3390/ijms13033145>.
- Kai, H., & Iba, K., (2014). *Temperature Stress in Plants* (p. a0001320). in: John Wiley & Sons Ltd (Ed.), ELS. John Wiley & Sons, Ltd, Chichester, UK. pub2. <https://doi.org/10.1002/9780470015902.a0001320.pub2>.
- Kamithi, K., Wachira, O., & Kid, A., (2016). Effects of different priming methods and priming duration on enzyme activities in germinating chickpea (*Cicer arietinum* L.). *Am. J. Nat. Appl. Sci.*
- Karimi, S., Eshghi, S., Karimi, S., & Hasan-Nezhadian, S., (2017). Inducing salt tolerance in sweet corn by magnetic priming. *Acta Agric. Slov.*, 109, 89–102.

- Kataria, S., & Jain, M., (2019). Magnetopriming alleviates the adverse effects of abiotic stresses in plants. In: Hasanuzzaman, M., Fujita, M., Oku, H., & Islam, M. T., (eds.), *Plant Tolerance to Environmental Stress* (1<sup>st</sup> edn., pp. 427–438). CRC Press, Boca Raton, FL.
- Kataria, S., & Verma, S. K., (2018). Salinity stress responses and adaptive mechanisms in major glycophytic crops: The story so far. In: Vinay, K., Shabir, H. W., Penna, S., & Lamson, P. T., (eds.), *Salinity Responses and Tolerance in Plants: Targeting Sensory, Transport and Signaling Mechanisms* (Vol. 1, pp. 1–39). Springer, Cham, Chapter-1.
- Kataria, S., Baghel, L., & Guruprasad, K. N., (2015). Effect of seed pretreatment by magnetic field on the sensitivity of maize seedlings to ambient ultraviolet radiation (280–400 nm). *Int. J. Trop. Agric.*, 33, 1–7.
- Kataria, S., Baghel, L., & Guruprasad, K. N., (2017a). Pre-treatment of seeds with static magnetic field improves germination and early growth characteristics under salt stress in maize and soybean. *Biocatal. Agric. Biotechnol.*, 10, 83–90.
- Kataria, S., Baghel, L., & Guruprasad, K. N., (2017b). Alleviation of adverse effects of ambient UV stress on growth and some potential physiological attributes in soybean (*Glycine max*) by seed pre-treatment with static magnetic field. *J. Plant Growth Regul.*, 36, 550–565.
- Kataria, S., Baghel, L., Jain, M., & Guruprasad, K. N., (2019). Magnetopriming regulates antioxidant defense system in soybean against salt stress. *Biocatalysis and Agricultural Biotechnology*, 18, 1878–1811. <https://doi.org/10.1016/j.bcab.2019.101090>.
- Kataria, S., Baroniya, S. S., Baghel, L., & Kanungo, M., (2014b). Effect of exclusion of solar UV radiation on plants. *Plant Sci. Today*, 1, 224–232.
- Kataria, S., Jain, M., Rastogi, A., & Brestic, M., (2021). Static magnetic field treatment enhanced photosynthetic performance in soybean under supplemental ultraviolet-B (280–320 nm) radiation. *Photosynthesis Res.* <https://doi.org/10.1007/s11120-021-00850-2>.
- Kataria, S., Jain, M., Tripathi, D. K., & Singh, V. P., (2020a). Involvement of nitrate reductase-dependent nitric oxide production in magnetopriming-induced salt tolerance in soybean. *Physiol. Plant.*, 168, 422–436.
- Kataria, S., Jajoo, A., & Guruprasad, K. N., (2014a). Impact of increasing ultraviolet-B radiation on photosynthetic processes. *J. Photochem. Photobiol. B*, 137, 55–66.
- Kataria, S., Rastogi, A., Bele, A., & Jain, M., (2020b). Role of nitric oxide and reactive oxygen species in static magnetic field pre-treatment induced tolerance to ambient UV-B stress in soybean. *Physiol. Mol. Biol. Plant.*, 26, 931–945.
- Kaur, S., Gupta, A. K., & Kaur, N., (2002). Effect of osmo- and hydropriming of chickpea seeds on seedling growth and carbohydrate metabolism under water deficit stress. *Plant Growth Regulation*, 37, 17–22. <https://doi.org/10.1023/A:1020310008830>.
- Kaya, M. D., Okçu, G., Atak, M., Çikili, Y., & Kolsarici, Ö., (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *European Journal of Agronomy*, 24(4), 291–295. <https://doi.org/10.1016/J.EJA.2005.08.001>.
- Kester, S., Geneve, R., & Houtz, R., (1997). Priming and accelerated ageing affect L-isoaspartyl methyltransferase activity in tomato (*Lycopersicon esculentum* Mill.) seed. *J. Exp. Bot.*, 48, 943–949.
- Khajeh-Hosseini, M., Powell, A. A., & Bimingham, I. J., (2003). The interaction between salinity stress and seed vigor during germination of soybean seeds. *Seed Sci. Technol.*, 31, 715–725.
- Khan, F., Hussain, S., Khan, S., & Geng, M., (2020). Seed priming improved antioxidant defense system and alleviated Ni-induced adversities in rice seedlings under N, P, or

- K Deprivation. *Front Plant Sci.*, 11, 565647. doi: 10.3389/fpls.2020.565647. PMID: 33013986; PMCID: PMC7509405.
- Knypl, J. S., & Janas, K. M., (1979). Increasing low-temperature resistance of soybean, *Glycine max* (L) Merr., by exposure of seeds to water-saturated atmosphere. *Biol. Plant Acad. Sc. Bohemoslov.*, 21, 291–297.
- Korkmaz, A., Korkmaz, Y., & Demirkiran, A., (2010). Enhancing chilling stress tolerance of pepper seedlings by exogenous application of 5-aminolevulinic acid. *Environ. Exp. Bot.*, 67, 495–501.
- Krylov, A., & Tarakanova, G. A., (1960). Magnetotropism of plants and its nature. *Plant Physiology*, 7, 156–160.
- Kumar, A., Gangwar, J. S., Prasad, S., & Harris, D., (2002). On-farm seed priming increases yield of direct-sown finger millet in India. *Int. Sorghum Millets News*, 43, 90–92.
- Kumar, M., Pant, B., Mondal, S., & Bose, B., (2016). Hydro and halo priming: Influenced germination responses in wheat var-HUW-468 under heavy metal stress. *Acta Physiol. Plant.*, 38, 217. <https://doi.org/10.1007/s11738-016-2226-3>.
- Laube, J., Sparks, T. H., Estrella, N., Heofler, J., Ankerst, D. P., & Menzel, A., (2014). Chilling outweighs photoperiod in preventing precocious spring development. *Glob. Change Biol.*, 20, 170–182.
- Lazaridou, M., & Noitsakis, B., (2003). The effect of water deficit on yield and water use efficiency of lucerne. In: *Optimal Forage Systems for Animal Production and the Environment; Proceedings of the 12<sup>th</sup> Symposium of the European Grassland Federation* (pp. 344–347). Pleven, Bulgaria. Bulgarian Association for Grassland and Forage Production (BAGFP).
- Lee -Suskoon, K., Hyeum, J., Beom, H., Minkyong, K., & Euiho, P., (1998). Optimum water potential, temperature and duration for priming of rice seeds. *J. Crop Sci.*, 43, 1–5.
- Lee, S., Moon, J. S., Domier, L. L., & Korban, S. S., (2002). Molecular characterization of phytochelatin synthase expression in transgenic *Arabidopsis*. *Plant Physiology and Biochemistry*, 40, 727–733. [https://doi.org/10.1016/S0981-9428\(02\)01430-4](https://doi.org/10.1016/S0981-9428(02)01430-4).
- Li, F., Wu, X., Tsang, E., & Cutler, A. J., (2005). Transcriptional profiling of imbibed *Brassica napus* seed. *Genomics*, 86, 718–730. <https://doi.org/10.1016/j.ygeno.2005.07.006>.
- Lobell, D. B., Schlenker, W., & Costa-Roberts, J., (2011). Climate trends and global crop production since 1980. *Science*, 333, 616–620. <https://doi.org/10.1126/science.1204531>.
- Lutts, S., Benincasa, P., Wojtyla, L., Kubala, S., Pace, R., Lechowska, K., Quinet, M., & Garnczarska, M., (2016). Seed priming: New comprehensive approaches for an old empirical technique. In: Araujo, S., & Balestrazzi, A., (eds.), *New Challenges in Seed Biology - Basic and Translational Research Driving Seed Technology*. InTech. <https://doi.org/10.5772/64420>.
- Manickavelu, A., Nadarajan, N., Ganesh, S., Gnanamalar, R., & Babu, R., (2006). Drought tolerance in rice: Morphological and molecular genetic consideration. *Plant Growth Regul.*, 50, 121–138.
- Matias, J. R., Torres, S. B., Leal, C. C. P., Leite, M. De. S., & Carvalho, S. M. C., (2018). Hydropriming as inducer of salinity tolerance in sunflower seeds. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 22(4), 255–260. <https://doi.org/10.1590/1807-1929/AGRIAMBI.V22N4P255-260>.
- Mauch-Mani, B., Baccelli, I., Luna, E., & Flors, V., (2017). Defense priming: An adaptive part of induced resistance. *Annu. Rev. Plant Biol.*, 68, 485–512. <https://doi.org/10.1146/annurev-arplant-042916-041132>.

- McDonald, M., (2000). Seed priming. In: Black, M., & Bewley, J. D., (eds.), *Seed Technology and its Biological Basis*. Sheffield Academic Press, Sheffield.
- Mohammadi, M., Dezfuli, P., & Sharifzadeh, F., (2008). Seed invigoration techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. *J. Appl. Plant Physiol.*, 34, 215–226.
- Mohammadi, R., & Roshandel, P., (2020). Ameliorative effects of a static magnetic field on hyssop (*Hyssopus officinalis* L.) growth and phytochemical traits under water stress. *Bioelectromagnetics*, 41(6), 403–412. doi: 10.1002/bem.22278. Epub 2020 Jun 22. PMID: 32573004.
- Monakhova, O. F., & Cherniad'ev, I. I., (2002). [Protective role of kartolin-4 in soil drought in wheat plants]. *Prikl. Biokhim. Mikrobiol.*, 38, 433–440.
- Mridha, N., Chattaraj, S., Chakraborty, D., Anand, A., Aggarwal, P., & Nagarajan, S., (2016). Pre-sowing static magnetic field treatment for improving water and radiation use efficiency in chickpea (*Cicer arietinum* L.) under soil moisture stress. *Bioelectromagnetics*, 37, 400–408.
- Munns, R., James, R. A., & Läuchli, A., (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*, 57, 1025–1043. <https://doi.org/10.1093/jxb/erj100>.
- Nadali, F., Asghari, H. R., Abbasdokht, H., Dorostkar, V., & Bagheri, M., (2020). Improved quinoa growth, Physiological response, and yield by hydropriming under drought stress conditions. *Gesunde Pflanzen*, 73(1), 53–66. <https://doi.org/10.1007/S10343-020-00527-1>.
- Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M., (2010). Heavy metals, occurrence and toxicity for plants: A review. *Environ. Chem. Lett.*, 8, 199–216. <https://doi.org/10.1007/s10311-010-0297-8>.
- Nagar, R., Dadlani, P., & Sharma, S., (1998). Effect of hydro-priming on field emergence and crop growth of maize genotypes. *Seed Sci. Res.*, 26, 1–5.
- Nam, N. H., Chauhan, Y. S., & Johansen, C., (2001). Effect of timing of drought stress on growth and grain yield of extra-short-duration pigeonpea lines. *J. Agric. Sci.*, 136, 179–189. <https://doi.org/10.1017/S0021859601008607>.
- Nascimento, J. P. B., Dantas, B. F., & Meiado, M. V., (2021). Hydropriming changes temperature thresholds for seed germination of tree species from the caatinga, a Brazilian tropical dry forest. *J. Seed Sci.*, 43, e202143004. <https://doi.org/10.1590/2317-1545v43238649>.
- Nawaz, A., Amjad, M., Jahangir, M. M., Khan, S. M., Cui, H., & Hu, J., (2012). Induction of salt tolerance in tomato (*Lycopersicon esculentum* Mill.) seeds through sand priming. *Aust. J. Crop Sci.*, 6(7), 1199–1203.
- Nawaz, A., Farooq, M., Ahmad, R., Basra, S. M. A., & Lal, R., (2016). Seed priming improves stand establishment and productivity of no till wheat grown after direct seeded aerobic and transplanted flooded rice. *Eur. J. Agron.*, 76, 130–137.
- Nerd, A., & Nobel, P., (1991). Effects of drought on water relations and nonstructural carbohydrates in cladodes of *Opuntia Ficus-indica*. *Physiol. Plant.*, 81, 495–500.
- Novitskaya, G. V., Molokanov, D. R., Kocheshkova, T. K., & Novitskii, Y. I., (2010). Effect of weak constant magnetic field on the composition and content of lipids in radish seedlings at various temperatures. *Russ. J. Plant Physiol.*, 57(1), 52–61.
- Nyakane, N. E., Markus, E. D., & Sedibe, E. D. M., (2019). The effects of magnetic fields on plants growth: A comprehensive review. *Int. J. Food Eng.*, 5, 79–87.
- Okçu, G., Kaya, M., & Atak, M., (2005). Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). *Turk J. Agric. For.*, 29, 237–242.

- Ozgun, R., Uzilday, B., Sekmen, A. H., & Turkan, I., (2013). Reactive oxygen species regulation and antioxidant defense in halophytes. *Functional Plant Biology*, 40, 832–847.
- Pandita, V. K., Anand, A., & Nagarajan, S., (2007). Enhancement of seed germination in hot pepper following presowing treatments. *Seed Sci. Technol.*, 35, 282–290. <https://doi.org/10.15258/sst.2007.35.2.04>.
- Paparella, S., Araújo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A., (2015). Seed priming: State of the art and new perspectives. *Plant Cell Rep.*, 34, 1281–1293. <https://doi.org/10.1007/s00299-015-1784-y>.
- Parida, A. K., & Das, A. B., (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicology and Environmental Safety*, 60, 324–349. <https://doi.org/10.1016/j.ecoenv.2004.06.010>.
- Parihar, P., Singh, S., Singh, R., Singh, V. P., & Prasad, S. M., (2015). Changing scenario in plant UVB research: UV-B from generic stressor to a specific regulator. *J. Photochem. Photobiol. B*, 153, 334–343.
- Parihar, P., Singh, S., Singh, R., Singh, V. P., & Prasad, S. M., (2015). Effect of salinity stress on plants and its tolerance strategies: A review. *Environ. Sci. Pollut. Res.*, 22, 4056–4075. <https://doi.org/10.1007/s11356-014-3739-1>.
- Patel, P., (2020). *Impact of Magneto-Priming of Seeds with Special Reference to Photosynthesis and Yield of Soybean and Maize*. Ph.D. Thesis, School of Life Sciences, Devi Ahilya University, Indore.
- Peuke, A. D., Schraml, C., Hartung, W., & Rennenberg, H., (2002). Identification of drought-sensitive beech ecotypes by physiological parameters. *New Phytologist.*, 154, 373–387. <https://doi.org/10.1046/j.1469-8137.2002.00400.x>.
- Plažek, A., Dubert, F., Kopeć, P., Dziurka, M., Kalandyk, A., Pastuszak, J., & Wolko, B., (2018). Seed hydropriming and smoke water significantly improve low-temperature germination of *Lupinus angustifolius* L. *International Journal of Molecular Sciences*, 19(4). <https://doi.org/10.3390/IJMS19040992>.
- Posmyk, M. M., & Janas, K. M., (2007). Effects of seed hydropriming in presence of exogenous proline on chilling injury limitation in *Vigna radiata* L. seedlings. *Acta Physiologiae Plantarum*, 29(6), 509–517.
- Prajapati, R., Kataria, S., & Jain, M., (2020). Seed priming for alleviation of heavy metal toxicity in plants: An overview. *Plant Science Today*, 7(3), 16. <https://doi.org/10.14719/pst.2020.7.3.751>.
- Radhakrishnan, R., & Kumari, B. D. R., (2013). Protective role of pulsed magnetic field against salt stress effects in soybean organ culture. *Plant Biosystems*, 147(1), 135–140. <http://dx.doi.org/10.1080/11263504.2012.717543>.
- Radhakrishnan, R., Leelapriya, T., & Kumari, B. D. R., (2012). Effects of pulsed magnetic field treatment of soybean seeds on Calli growth, cell damage, and biochemical changes under salt stress. *Bio Electro Magnetism*, 33(8), 670–681. <https://doi.org/10.1002/bem.21735>.
- Rai, V., Vajpayee, P., Singh, S. N., & Mehrotra, S., (2004). Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. *Plant Science*, 167, 1159–1169. <https://doi.org/10.1016/j.plantsci.2004.06.016>.
- Raipuria, R. K., Kataria, S., Watts, A., & Jain, M., (2021). Magneto-priming promotes nitric oxide via nitric oxide synthase to ameliorate the UV-B stress during germination of soybean seedlings. *Journal of Photochemistry and Photobiology B: Biology*, 220, 112211. <https://doi.org/10.1016/j.jphotobiol.2021.112211>.



- Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C., & Job, D., (2012). Seed germination and vigor. *Annu. Rev. Plant Biol.*, 63, 507–533. <https://doi.org/10.1146/annurev-arplant-042811-105550>.
- Rashid, A., Hollington, P. A., Harris, D., & Khan, P., (2006). On-farm seed priming for barley on normal, saline and saline-sodic soils in North West Frontier Province, Pakistan. *Eur. J. Agron.*, 24, 276–281.
- Rathod, G. R., & Anand, A., (2016). Effect of seed magneto-priming on growth, yield and Na/K ratio in wheat (*Triticum aestivum* L.) under salt stress. *Ind. J. Plant Physiol.*, 21, 15–22. <https://doi.org/10.1007/s40502-015-0189-9>.
- Razmjoo, J., & Alinian, S., (2017). Influence of magnetopriming on germination, growth, physiology, oil and essential contents of cumin (*Cuminum cyminum* L.). *Electromagnetic Biology and Medicine*, 36, 325–329. <https://doi.org/10.1080/15368378.2017.1373661>.
- Renugadevi, J., & Vijayageetha, V., (2013). Organic seed fortification in cluster bean (*Cyamopsis tetragonoloba* L.) *Taub*, 335–337.
- Roy, N., & Srivastava, A. K., (1999). Effect of presoaking seed treatment on germination and amylase activity of wheat (*Triticum aestivum* L.) under salt stress conditions. *Rachis*, 18, 46–51. Barley and Wheat Newsletter.
- Rozema, J., Bjorn, L. O., Bornman, J. F., Gaberscik, A., Häder, D. P., Trost, T., Germ, M., et al., (2002). The role of UV-B radiation in aquatic and terrestrial ecosystems—An experimental and functional analysis of the evolution of UV-absorbing compounds. *J. Photochem. Photobiol. B*, 66, 2–12.
- Ruzic, R., & Jerman, I., (2002). Weak magnetic field decreases heat stress in cress seedlings. *Electromagn. Biol. Med.*, 21, 69–80.
- Samarah, N. H., (2005). Effects of drought stress on growth and yield of barley. *Agron. Sustain. Dev.*, 25, 145–149. <https://doi.org/10.1051/agro:2004064>.
- Sarraf, M., Kataria, S., Taimourya, H., Oliveira, L. S., Menegatti, R. D., Jain, M., Ihtisham, M., & Liu, S., (2020). Magnetic field (MF) applications in plants: An overview. *Plants*, 9, 1139. <https://doi.org/10.3390/plants9091139>.
- Schutzendubel, A., (2002). Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany*, 53, 1351–1365. <https://doi.org/10.1093/jexbot/53.372.1351>.
- Sebastiani, L., Scebbba, F., & Tognetti, R., (2004). Heavy metal accumulation and growth responses in poplar clones eridano (*Populus deltoides* × *maximowiczii*) and I-214 (*P. × euramericana*) exposed to industrial waste. *Environmental and Experimental Botany*, 52, 79–88. <https://doi.org/10.1016/j.envexpbot.2004.01.003>.
- Selim, A. F. H., & El-Nady, M. F., (2011). Physio-anatomical responses of drought stressed tomato plants to magnetic field. *Acta Astronaut.*, 69, 387–396.
- Sen, A., & Alikamanoglu, S., (2014). Effects of static magnetic field pretreatment with and without PEG 6000 or NaCl exposure on wheat biochemical parameters. *Russ J. Plant Physiol.*, 61(5), 646–655.
- Sen, A., & Puthur, J. T., (2020). Influence of different seed priming techniques on oxidative and antioxidative responses during the germination of *Oryza sativa* varieties. *Physiology and Molecular Biology of Plants: An International Journal of Functional Plant Biology*, 26(3), 551–565. <https://doi.org/10.1007/s12298-019-00750-9>.
- Shabrangy, A., Ghatak, A., Zhang, S., Priller, A., Chaturvedi, P., & Weckwerth, W., (2021). Magnetic field induced changes in the shoot and root proteome of barley (*Hordeum vulgare* L.). *Front. Plant Sci.*, 12, 622795. doi: 10.3389/fpls.2021.622795.

- Shafi, M., Bakht, J., Hassan, M. J., Raziuddin, M., & Zhang, G., (2009). Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (*Triticum aestivum* L.). *Bull Environ. Contam. Toxicol.*, 82, 772–776. <https://doi.org/10.1007/s00128-009-9707-7>.
- Shannon, M. C., & Grieve, C. M., (1998). Tolerance of vegetable crops to salinity. *Scientia Horticulturae*, 78, 5–38. [https://doi.org/10.1016/S0304-4238\(98\)00189-7](https://doi.org/10.1016/S0304-4238(98)00189-7).
- Sharma, S. S., & Dietz, K. J., (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany*, 57, 711–726. <https://doi.org/10.1093/jxb/erj073>.
- Shine, M. B., & Guruprasad, K. N., (2012a). Impact of presowing magnetic field exposure of seeds to stationary magnetic field on growth, reactive oxygen species and photosynthesis of maize under field conditions. *Acta Physiol. Plant.*, 34, 255–265.
- Shine, M. B., & Guruprasad, K. N., (2012b). Oxyradicals and PS II activity in maize leaves in the absence of UV components of solar spectrum. *J. Biosci.*, 37, 703–712.
- Shine, M. B., Guruprasad, K. N., & Anand, A., (2012). Enhancement of germination, growth, and photosynthesis in soybean by pre-treatment of seeds with magnetic field. *Bioelectromagnetics*, 32(6), 474–484.
- Siddique, M. R. B., Hamid, A., & Islam, M. S., (2000). Drought stress effects on water relations of wheat. *Bot. Bull. Acad. Sin.*, 41, 35–39.
- Singh, H. P., Mahajan, P., Kaur, S., Batish, D. R., & Kohli, R. K., (2013). Chromium toxicity and tolerance in plants. *Environ. Chem. Lett.*, 11, 229–254. <https://doi.org/10.1007/s10311-013-0407-5>.
- Srivastava, A. K., Lokhande, V. H., Patade, V. Y., Suprasanna, P., Sjahril, R., & D'Souza, S. F., (2010). Comparative evaluation of hydro-, chemo-, and hormonal-priming methods for imparting salt and PEG stress tolerance in Indian mustard (*Brassica juncea* L.). *Acta Physiol. Plant.*, 32, 1135–1144. <https://doi.org/10.1007/s11738-010-0505-y>.
- Sugie, A., Naydenov, N., Mizuno, N., Nakamura, C., & Takumi, S., (2006). Overexpression of wheat alternative oxidase gene Waox1a alters respiration capacity and response to reactive oxygen species under low temperature in transgenic *Arabidopsis*. *Genes Genet. Syst.*, 81, 349–354. <https://doi.org/10.1266/ggs.81.349>.
- Sung, J., & Chiu, K., (1995). Hydration effect on seedling emergence strength of watermelon seeds differing in ploidy. *Plant Sci.*, 110, 21–26.
- Suzuki, N., & Mittler, R., (2006). Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. *Physiol. Plant.*, 126, 45–51. <https://doi.org/10.1111/j.0031-9317.2005.00582.x>.
- Taylor, A. G., Allen, P. S., Bennett, M. A., Bradford, K. J., Burris, J. S., & Misra, M. K., (1998). Seed enhancements. *Seed Sci. Res.*, 8, 245–256. <https://doi.org/10.1017/S0960258500004141>.
- Thakur, M., Sharma, P., & Anand, A., (2019). Seed priming-induced early vigor in crops: An alternate strategy for abiotic stress tolerance. In: Hasanuzzaman, M., & Fotopoulos, V., (eds.), *Priming and Pretreatment of Seeds and Seedlings*. Springer, Singapore. [https://doi.org/10.1007/978-981-13-8625-1\\_8](https://doi.org/10.1007/978-981-13-8625-1_8).
- Thakur, M., Sharma, P., Anand, A., Pandita, V. K., Bhatia, A., & Pushkar, S., (2020). Raffinose and hexose sugar content during germination are related to infrared thermal fingerprints of primed onion (*Allium cepa* L.) seeds. *Front. Plant Sci.*, 11, 579037. <https://doi.org/10.3389/fpls.2020.579037>.

- Thomas, S., Anand, A., Chinnusamy, V., Dahuja, A., & Basu, S., (2013). Magnetopriming circumvents the effect of salinity stress on germination in chickpea seeds. *Acta Physiol. Plant.*, 35, 3401–3411.
- Thornton, J. M., Collins, A. R. S., & Powell, A. A., (1993). The effect of aerated hydration on DNA synthesis in embryos of *Brassica oleracea* L. *Seed Science Research*, 3, 195–199. <https://doi.org/10.1017/S0960258500001781>.
- Turunen, M., & Latola, K., (2005). UV-B radiation and acclimation in timberline plants. *Environ. Pollut.*, 137, 390–403.
- Valko, M., Morris, H., & Cronin, M., (2005). Metals, toxicity and oxidative stress. *CMC*, 12, 1161–1208. <https://doi.org/10.2174/0929867053764635>.
- Vari, A., Mitrabinda, S., Dadlani, M., & Sharma, S. P., (2003). Physiological and biochemical changes associated with osmopriming in maize seeds. In: *2<sup>nd</sup> International Congress of Plant Physiology*. New Delhi, India.
- Varier, A., Vari, A., & Dadlani, M., (2010). The sub cellular basis of seed priming. *Curr. Sci.*, 99, 450–456.
- Vashisth, A., & Nagarajan, S., (2010). Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. *J. Plant Physiol.*, 167, 149–156.
- Viehweger, K., (2014). How plants cope with heavy metals. *Bot. Stud.*, 55, 35. <https://doi.org/10.1186/1999-3110-55-35>.
- Vollenweider, P., & Günthardt-Goerg, M. S., (2005). Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. *Environmental Pollution*, 137, 455–465. <https://doi.org/10.1016/j.envpol.2005.01.032>.
- Wahid, A., Rasul, E., Rao, R., & Iqbal, R., (2005). Photosynthesis in leaf, stem, flower and fruit. *Handb. Photosynth*, 2, 479–497.
- Walter, J., Jentsch, A., Beierkuhnlein, C., & Kreyling, J., (2013). Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environmental and Experimental Botany*, 94, 3–8. <https://doi.org/10.1016/j.envexpbot.2012.02.009>.
- Wang, W., Vinocur, B., & Altman, A., (2003). Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, 218, 1–14. <https://doi.org/10.1007/s00425-003-1105-5>.
- Warren, J., & Bennett, M. A., (1997). Seed hydration using the drum priming system. *Hort. Sci. Hort. Sci.*, 32, 1220, 1221.
- Wechsberg, G., Probert, R., & Bray, C., (1995). The relationship between ‘dehydrin like’ protein and seed longevity in *Ranunculus sceleratus* L. *J. Exp. Bot.*, 45, 1027–1030.
- Weitbrecht, K., Muller, K., & Leubner-Metzger, G., (2011). First off the mark: Early seed germination. *J. Exp. Bot.*, 62, 3289–3309. <https://doi.org/10.1093/jxb/err030>.
- Wong, H. L., Sakamoto, T., Kawasaki, T., Umemura, K., & Shimamoto, K., (2004). Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. *Plant Physiol.*, 135, 1447–1456. <https://doi.org/10.1104/pp.103.036384>.
- Xi, G., Fu, Z. D., & Ling, J., (1994). Change of peroxidase activity in wheat seedlings induced by magnetic field and its response under dehydration condition. *Acta Bot. Sinica*, 36, 113–118.
- Xiong, L., & Zhu, J. K., (2002). Molecular and genetic aspects of plant responses to osmotic stress: Plant responses to osmotic stress. *Plant, Cell & Environment*, 25, 131–139. <https://doi.org/10.1046/j.1365-3040.2002.00782.x>.

- Yagmur, M., & Kaydan, D., (2008). Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. *Afr. J. Biotechnol.*, 7(13), 2156–2162.
- Zeid, I. M., & Shedeed, Z. A., (2006). Response of alfalfa to putrescine treatment under drought stress. *Biologia Plant.*, 50, 635–640. <https://doi.org/10.1007/s10535-006-0099-9>.
- Zheng, M., Tao, Y., Hussain, S., Jiang, Q., Peng, S., Huang, J., Cui, K., & Nie, L., (2016). Seed priming in dry direct-seeded rice: Consequences for emergence, seedling growth and associated metabolic events under drought stress. *Plant Growth Regul.*, 78, 167–178. <https://doi.org/10.1007/s10725-015-0083-5>.



# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

## CHAPTER 5

---

# Magneto-Priming: A Novel Technique Towards Improved Seed Germinability and Stress Responses: From Basics to Contemporary Advancements

KUNTAL BERA,<sup>1,2</sup> SUBIR GHOSH,<sup>2</sup> SANJOY SADHUKHAN,<sup>2</sup> and PUSPENDU DUTTA<sup>1\*</sup>

*<sup>1</sup>Department of Seed Science and Technology, Uttar Banga Krishi Viswavidyalaya, Pundibari – 736165, Cooch Behar, West Bengal, India*

*<sup>2</sup>Plant Molecular Biology Laboratory, Department of Botany, Raiganj University, Raiganj – 733134, Uttar Dinajpur, West Bengal, India*

*\*Corresponding author. E-mail: pdutta.pph@gmail.com*

---

### ABSTRACT

The present era of precision agriculture demands that every seed will germinate and develop into a healthy plant. Improved emergence and uniform crop establishment in the field are the most critical that determine the final yield. But seed germination and early seedling growth are often impeded due to the frequent occurrence of sub-optimal conditions, particularly under the context of global climate change. Seed priming has been recognized long before as an effective technique that can potentially improve seed germination, ensure good plant establishment, and ultimately result in enhanced crop productivity in a wide range of environments. Nowadays, the physical means of seed priming are extensively being explored due to having several advantages over conventional seed priming methods as the later ones mostly rely on restricted hydration of the seed until the germination process starts and raise

an issue of reduced storability during post-priming periods. Furthermore, being eco-friendly, exploitation of physical methods would offer a unique option towards agricultural sustainability.

Among the physical means, magneto-priming is not only a non-ionizing and non-invasive method but also an affordable dry seed priming treatment and a novel technique with proven positive influences on seed germination and the growth or yield of crops. Magneto-priming basically includes two types of pre-sowing treatment either with the static magnetic field (SMF) or electromagnetic field (EMF). Magneto-priming generally involves pre-exposure of seed to magnetic fields with values greater than the earth's geomagnetic field. Apart from having stimulatory effects on seed germination and crop growth, seed pre-treatment with magnetic fields has been reported to favorably alter several physiological attributes and biochemical processes. Moreover, magneto-priming can be used as a non-lethal physical stimulant to the seed as it develops a stress memory within the seed. The imprint of this priming memory is effectively carried to the subsequent growth stages of crop plants, and as such it helps to build up protection against different kinds of abiotic stresses. Though the underlying mechanisms involved in magneto-priming induced enhancement in germination or plant growth are not well-understood but the changes in electronic configuration, energy transformation, activation, and regulation in the kinetics of several biochemical pathways are thought to be the major reasons behind the positive responses achieved under magneto-priming. With this background, this chapter aims to elucidate the basics of magneto-priming and the possibilities of exploiting this technique in improving the production, or the stress responses of crop plants. Further, efforts have been made to summarize recent progress in underlying mechanisms involved in magneto-priming.

## **5.1 INTRODUCTION**

The agricultural production system needs to keep up the pace of increasing rate of crop yield for food security of the ever-rising global population. Thus, scientists are constantly in search of promising technologies that are not only economical and eco-friendly rather have the potential to improve germination, seedling vigor and ultimately enhance crop yield. Seed priming, including both chemical and physical methods, is one of the technologies that can be exploited to enhance seed quality and its field performance. However, the physical techniques of seed priming have been revealed to

be beneficial in various aspects over the conventional means. Among the various approaches, seed pretreatment with a magnetic field has been recognized as a novel and non-invasive dry seed priming procedure with proven beneficial influence on radicle emergence and seedling vigor in a variety of crops. In recent years, many researchers have reported that seed magnetization with low-to-medium level magnetic field (MF) can favorably alter metabolic activities that occur during seed germination and early seedling stage (Anand et al., 2019; Bhardwaj et al., 2012; Shine et al., 2011; Sunita et al., 2015). Further, the application of biophysical factors like MF would lower the risk of the generation of toxins in plants or plant products leading to food and environmental protection (Aladjadjiyan, 2007). By comprehending the various interactions between EMF and physio-biochemical processes in plants, MF has been found to be a feasible alternative to chemical-based approaches of seed priming (Aladjadjiyan, 2002; Belyavskaya, 2004; Da Silva & Dobránszki, 2016).

The extreme application of EMFs with the progress of various domains of science has changed the world over the last few decades and has raised questions on the biological effects of this field. Plants are exposed to MF to a greater extent than any other species due to the extensive and excessive use of magnets like different kinds of static magnets and different electromagnets in domestic appliances, medical equipment, industrial equipment, vehicles, and communication devices, etc. (Athari et al., 2008). Further, with such technological advancements, the strength of the geomagnetic field is rising beyond the level of 50–60  $\mu\text{T}$  that can affect seed germination and growth of plants (Zhadin, 2001). Thus, plants development would either be positively or negatively affected as a consequence of such constant exposure to varying strength or intensity of natural MF (Kordas, 2002).

The effect of MF on plants was first recorded by Krylov (1960). They were also the first to suggest the term magnetotropism ascribing the auxin-like effect of MFs on seed germination. The observation on the root-growth response of a variety of plants suggests that some intrinsic factors must have been involved before the manifestation of tropism (Pittman, 1962). Such opinions have also led to the thought that the root-growth response of plants would be magnetotropic or geo-magnetotropic rather than directly heliotropic. In the last 10 to 15 years, plant science has made significant progress in understanding the impact of MF on seed germination and subsequent growth or development of crop plants. It is now evident that pre-sowing exposure of seeds to the magnetic field reduces planting costs by significantly increasing germination rates as well as establishing good plant stand



by promoting growth (Efthimiadou et al., 2014; Mahajan & Pandey, 2014; Menegatti et al., 2019). However, contradictory results of growth inhibition have also been reported under exposure of MF (Abdolmaleki et al., 2007; Ijaz et al., 2012). The benefit of using MF in improving germination rates has been ascribed as the possible increase in membrane permeability that results in faster water absorption by seed (Reina & Pascual, 2001). Few studies also suggest that MF would influence water and nutrient absorption, and thereby promote the growth of plants (Hilal et al., 2002; Maheshwari & Grewal, 2009). Along with improving germination rates, pre-sowing exposure of seeds to MF would bring about several positive effects including increased cell proliferation potential that lead to rapid plant growth (Cakmak et al., 2010). Pre-treating seeds with a magnetic field (MF) shows increased plant production, and also the negative effects of environmental extremities on plant growth and development have been lowered (Kataria & Jain, 2019; Prajapati et al., 2020; Sarraf et al., 2020). The impacts of magnetic stimulus on seeds under saline stress using static MF have been described by Thomas et al. (2013); and Kataria et al. (2017a). Seed pre-magnetization with a static magnetic field (SMF) has also been revealed to minimize the damaging effects of various stresses, i.e., heat (Ružič & Jerman, 2002), salinity (Thomas et al., 2013), ambient UV-B (Kataria et al., 2020), and cadmium (Chen et al., 2011). Apart from improving seed germination and seedling vigor, many studies also indicate that MF would not only enhance photosynthetic pigments, light energy absorption or photosystem II (PSII) efficiency, photosynthesis rate rather eliminate the negative impacts of various environmental stresses in soybean (Baghel et al., 2016; Kataria et al., 2017a, b, 2019). Furthermore, Mahajan & Pandey (2014) have concluded that the seed priming technique with the use of a magnetic field would be a feasible option to avoid agricultural pests and diseases, and thereby reduce the use of insecticides and pesticides in agriculture. MF has also been reported to increase the activity of antioxidant enzymes like peroxidase (POX), polyphenol oxidase (PPO), superoxide dismutase (SOD), and catalase (CAT) within plant cells, and these ROS scavenging enzymes can minimize the oxidative damage caused by free radical ions (Dhawi, 2014; Maffei, 2014; Vian et al., 2016). Though the application of EMFs would also trigger oxidative stress in the plant (Shabrangi & Majd, 2009). As a consequence of oxidative stress, high-energy electrons are shifted to molecular oxygen to generate reactive oxygen species (ROS); for example, singlet oxygen, superoxide ions, and peroxides. Thus, despite delivering several beneficial impacts on the plant, the reports on the negative effects of MF cannot be overlooked. The harmful effects of

MF include a decrease in cell growth, an increase in lignin and suberin on cell walls, or a decrease in seed germination and organ growth (Sahebamei et al., 2007).

Thus, it is now well established that all living beings including plants are always being exposed to the Earth's MF. However, unlike phototropism, gravitropism, and thigmotropism of plants that describe plant's response to various wavelengths of light, gravity, and touch or electrical signaling, respectively, the influence of MF on plant growth and development is not thoroughly studied or not well-understood. Under this context, the present chapter has been conceived to summarize the possibility of exploiting magneto-priming as a novel, safe, and cost-effective seed enhancement technique. Further, it intends to elucidate the current state of understanding of the physiological and molecular mechanisms behind the magneto-priming-induced alternation in plant systems.

## **5.2 MAGNETO-PRIMING AND PLANT RESPONSES**

The Earth's magnetic (i.e., geomagnetic) field is an inevitable environmental factor, and it varies significantly in strength and direction that keeps changing with the location. While the majority of the MF found on the Earth's surface has an internal source and it is primarily caused by the dynamo motion of turbulent flows in the planet's fluid metallic exterior core while the external MF due to ionosphere and magnetosphere plays a minor role (Qamili et al., 2013). The existence of a geomagnetic field helps to protect our planet and its environment from the influence of solar storm by bouncing most of the charged particles via the magnetosphere (Occhipinti et al., 2014). At the Earth's surface, the highest strength of vertical extension ( $67 \mu\text{T}$ ) and horizontal extension ( $33 \mu\text{T}$ ) of GMF is found at the magnetic pole and the magnetic equator, respectively (Kobayashi et al., 2004). Besides the existence of the Earth's magnetic field, the significant presence of a magnetic field has also been detected in and around all other celestial objects like galaxies ( $\leq 0.1 \text{ nT}$ ), close to the sun ( $0.21 \text{ nT}$ ) and the surface of Venus is  $3 \text{ nT}$  (Belov & Bochkarev, 1983). The geomagnetic field has certain consequences on the living organisms since all living components of the Earth are constantly being exposed to the inescapable geomagnetic field, and it has been reported that relatively low intensity of magnetic field can effectively stimulate or trigger plant growth responses. This indicates that the influence of the Earth's magnetic field on biological processes is not only debatable rather an interesting area of research. Therefore, studies on the impact of

magnetic fields with low and intermediate frequencies on biological components have been attractive to the researchers in recent times, and this has created a new wave of fervor among researchers on how the magnetic field plays a role in plant growth and development. Thus, magneto-priming is now gaining importance as effective and environment friendly technology-supported physical treatments of seeds. It is one of the advanced techniques of agronomical importance since magnetic field exposure brings out various positive influences such as increased germination rate and seed vigor, higher growth, enhanced crop yield as well as improved tolerance to abiotic stresses.

### 5.2.1 GERMINATION

The application of a magnetic field can break the dormancy of seeds, and subsequently improves the growth rate of different crop species like barley, maize, beans, wheat, and some fruit or other tree species. Further, a magnetic field of 16 Hz has been reported to be an effective method for improving the post-harvest quality of seeds of diverse plant species, specifically for low-temperature sensitive germinating seeds species (Rochalska & Orzeszko-Rywka, 2005).

Hornwort (*Cryptotaenia japonica*) showed higher cellular function and enzymatic activity during the germination stage when the seeds were exposed to ultra-low frequency magnetic field alongside planetary GMF varying with sinusoidal time. This leads to assuming the possibility of the existence of optimum extremely low-frequency MF within the planetary geomagnetic field for improved germination of hornwort seeds (Kobayashi et al., 2004). The outcomes particularly in terms of germination percentage, emergence index or vigor index have been found to be satisfactory when pea seeds (*Pisum sativum*) are treated with the rectified sinusoidal magnetic field of varying strength 60, 120, and 180 mT for three fixed time periods of 5, 10, and 15 minutes (Jamil & Ahmad, 2012). A significant upregulation of germination magnetic constant or other traits like transition time and water uptake along with an indication of the possibility of off-season germination has been recorded upon exposure of mungbean (*Vigna radiata*) seeds to the increasing intensity of static MF ranging from 87 to 226 mT for 1 hour and 40 mins (Mahajan & Pandey, 2014).

The exposure of chickpea (*Cicer arietinum*) seeds to an MF strength ranging between 0 mT and 250 mT for 1 to 4 hours at an interval of 50 mT has recorded a significant increase in germination speed along with the

seedling length and dry weight. Further, better performance of magnetoprimed seeds has been recorded under rain-fed conditions as compared to control (Vashisth & Nagarajan, 2008). In an experiment, Payez et al. (2013) soaked the wheat seeds in water overnight and then treated them with a 30 mT static magnetic field and 10 kHz of EMF for 5 hours a day and continued for 4 days. They observed a positive impact of both electromagnetic and static magnetic exposure on germination parameters and membrane integrity of seeds during germination.

Further, pulsed electromagnetic field (PMF) exposures on maize (*Zea mays*) seeds for various time periods viz., 0, 15, 30, and 45 mins have shown promotional effects on various germination and morpho-physiological parameters during seedling growth. It has also indicated that magnetic exposure for 30 and 45 mins would result in higher crop yield and the possibility of exploitation of magnetic fields as modern agricultural tools towards the economical development of farmers (Bilalis et al., 2012). A recent experiment also supports the beneficial effect of magnetopriming on maize (*Zea mays*) under salt stress conditions. The experimental design for seed pretreatment had been established by 200 mT of SMF for a period of 1 hour. The primed seeds showed a significant enhancement in germination percentage and stress tolerance with promotional effects on other growth and development related parameters (Baghel et al., 2019). Later on, Alvarez et al. (2021) observed the impact of SMF on Triticale seeds under osmotic and saline stress conditions while the seeds were treated with 3.71 mT of SMF for 10 hours against the control. This experiment yielded improved mean germination time and time required for 50% emergence. The effects of electromagnets on tomato (*Solanum lycopersicum*) cv. Lignon were studied by pretreatment of seeds to various combinations of rectified electromagnets, i.e., 80, 120, 160, and 200 mT for 1, 3, 5, 10, 15, and 20 mins. The results indicated that there was an increase in germination percentage with a decline in time required to germinate, increase in shoot, and root length under combined exposure of 1 min each to magnetic fields of 160 mT and 200 mT (De Souza et al., 2010). Further, Poinapen et al. (2013) reported that pre-exposure of seeds to MF resulted in an increase in germination percentage and plant growth. Further, soybean seeds (*Glycine max*) recorded a significantly higher germination rate when the seeds were pre-treated with a magnetic field of 0.002 mT at 10 and 100 Hz for 5 hours/day for 20 days (Radhakrishnan & Kumari, 2013). The pulsed EMF also has several promotional effects on broad beans including germination percentage. When seeds were primed with 12.5 mT pulsed EMF with 3 Hz frequency for 15 minutes, the germination percentage increased

significantly. The plants grown from treated seeds had been observed to have massive vegetative growth, but no significant effect was recorded in terms of reproductive yield (Katsenios et al., 2020).

In another study, Razmjoo & Alinian (2017) treated cumin seeds to a static magnetic field of varying strengths, i.e., 0, 150 mT, 250 mT, 500 mT, and 1 T for 12 min. An enhancement in germination attributes and early growth parameters was observed in all the treatments. They also reported that the days to emergence as well as flowering and maturity had been shortened along with increased chlorophyll and declined proline contents under exposure to static magnetic field. Braga et al. (2020) evaluated the impact of MF pretreatment on the germination of coffee seeds and hence, the seeds were treated with the magnetic stimulus of 10 mT and 28 mT for 6 days. They suggested that seed pre-magnetization process would be an inexpensive and effective technique since there was an improvement in the germination uniformity along with permeability of cellular membrane and activation of antioxidant systems under exposure to both magnetic field intensities. The exposure of an extremely low EMF of 0.03  $\mu$ T with 10 Hz frequency for 30 minutes was found to improve germination percentage with a higher accumulation of antioxidant enzymes in germinating Foxtail millet seeds (*Setaria italica*). The EMF treated seeds were recorded to enhance the germination percentage by 93% with a 15.66% higher germination efficiency than the untreated seeds (Ramesh et al., 2020). The impacts of magneto-priming on germination and subsequent seedling growth, or associated changes have been summarized and presented in Table 5.1.

### 5.2.2 IMPACTS ON PLANT GROWTH AND DEVELOPMENT

The germination and early seedling developmental stages are very critical since these two stages ultimately determine the overall growth and development of crop plants and ultimately its productivity. In the context of current challenges for agricultural sustainability and food security, optimizing the exposure duration and dose or intensity of physical agents is very pertinent. As such the researchers are continuously engaged to find out the impact of physical treatments including magnetic exposure on crop growth particularly during early stages.

The growth of the cucumber (*Cucumis sativus*) plant was found to increase significantly when magnetically treated water was used for irrigation during the cultivation of seeds (Hirota et al., 1999). Further, magnetized

**TABLE 5.1** Effects of Magneto-Priming on Seed Germination and Crop Performance

Crops	Dose of Magnetic Field	Effect	References
<i>Glycine max</i>	1.5 $\mu$ T at 10.0 Hz PMF for 5 h per day for 20 days	Increased activities of $\beta$ -amylase, CAT, PPO, and acid phosphatase, content of various minerals, plant height, leaf number, total number of pods, pod length, number of seeds, seed weight.  But reduced activities of hydrolytic enzymes as well as nitrate reductase enzyme or content of Ca.	Radhakrishnan & Kumari (2012)
<i>Helianthus annuus</i>	20 $\mu$ T	Increased fresh weight of seedlings	Fischer et al. (2004)
<i>Triticum aestivum</i>	From 0.02 $\mu$ T to 100 $\mu$ T	Increased activation rate of esterases but reduced plant growth	Aksyonov et al. (2007); Bogatina et al. (1978)
<i>Abelmoschus esculentus</i>	99 mT	Improved germination rate	Naz et al. (2012)
<i>Allium ascalonicum</i>	7 mT	Higher accumulation of $H_2O_2$ with increased lipid peroxidation rate	Cakmak et al. (2012)
<i>Coffea arabica</i>	2 mT	Decreased APX, SOD, and CAT activities	Alemán et al. (2014)
<i>Cucumis sativus</i>	100–250 mT	Increased generation of superoxide radicals and hydrogen peroxide	Bhardwaj et al. (2012)
<i>Glycine max</i>	1,500 nT of PMF for 20 days @ 10 Hz for 5 hrs. day <sup>-1</sup>	Reduced ROS generation but increased the Rubisco activity	Radhakrishnan & Kumari (2012)
<i>Triticum aestivum</i>	4 or 7 mT 30-mT	Increased germination rate, increased CAT activity but reduced peroxidase activity, changed the position of amyloplasts within the cells	Hasenstein et al. (2013); Payez et al. (2013)
<i>Vicia faba</i>	15 mT to 30 mT	Higher accumulation of ROS, the modified activity of catalase and MAPK	Haghighat et al. (2014); Jouni et al. (2012)
<i>Hyssopus officinalis</i>	90 mT, 200 mT for 5 min	Reduced electrolyte leakage and MDA content but increased CAT and APX activities, polyphenols, total chlorophyll contents and biomass at 200 mT	Mohammadi & Roshandel (2020)
<i>Vigna radiata</i> (Linn.) Wilczek.	87–226 mT SMF	Increased mean germination rate, imbibition rate with increasing intensity of MF	Mahajan & Pandey (2014)

**TABLE 5.1** (Continued)

Crops	Dose of Magnetic Field	Effect	References
<i>Passiflora edulis</i> Sims.	200 mT SMF	Increased rate of germination, seedling emergence and seedling vigor	Menegatti et al. (2019)
<i>Cucumis sativus</i> L.	200 mT SMF	Increased activities of hydrolytic enzymes, accumulation of ROS as well as antioxidant enzymes	Bhardwaj et al. (2012)
<i>Glycine max</i> (Linn.) Merr.	150–200 mT SMF	Increased rate of seed germination, biomass accumulation, number of root nodules, nitrogen metabolism, pigment synthesis, rate of photosynthesis and productivity	Baghel et al. (2016); Kataria et al. (2020); Shine et al. (2011)
<i>Solanum lycopersicum</i> Mill.	50–332 mT SMF	Germination rate increased, observed changes in biochemical and molecular level involving homeostasis of H <sub>2</sub> O <sub>2</sub> with improved seed vigor	Anand et al. (2019); Poinapen et al. (2013)
<i>Zea mays</i> L.	200 mT SMF	Improved seed germination and early growth of seedlings, enhanced activities of $\alpha$ -amylase, protease	Kataria et al. (2017b); Vashisth & Joshi (2017)
<i>Raphanus sativus</i> L.	8–20 mT SMF	Enhanced rate of germination and seedling vigor index	Konefał-Janocha et al. (2018)
<i>Capsicum annuum</i> L.	57–60 mT SMF	Enhanced seed germination, seedling growth, fruit yield as well as improved fruit quality	Ahamed et al. (2013)
<i>Hordeum vulgare</i> L.	35 mT SMF	Increased rate of seed germination and better plant establishment both under salinity stress and normal condition	Hozayn et al. (2018)
<i>Oryza sativa</i> L.	125–250 mT	Enhanced germination dynamics, increased root, and stem length	Florez et al. (2019)
<i>Phaseolus vulgaris</i> L.	4–7 mT, 130 mT	Increased GPOX activities in leaves and promoted mitotic activity in meristematic cells	Cakmak et al. (2010); Mroczek-Zdyrska et al. (2021)
<i>Tagetes patula</i>	100 mT	Enhanced germination, seedling vigor and starch metabolism	Afzal et al. (2012)

**TABLE 5.1** (Continued)

Crops	Dose of Magnetic Field	Effect	References
<i>Solanum melomgena</i> L.	MTW	Neutralized the soil pH value, yield increased on an average of 2.47% per unit area	Kareem (2018)
<i>Vicia faba</i> L.			
<i>Solanum lycopersicon</i> L.			
<i>Lens culinaris</i> Medik.	110 mT MW	Increased activity of APX but reduced SOD activity	Azimi et al. (2018)
<i>Moringa oleifera</i> Lam.	MTW (30 mT)	Improved growth under salt stress	Hasan et al. (2019)
<i>M. peregrina</i> (Forssk. Fiori)			
<i>Brassica rapa</i> L. var. <i>glabra</i> Regel.	MTW (211 mT)	Reduced electrical conductivity or total dissolved solids by 15.60% after 300 min of treatment, and also reduced the soil-soluble Na <sup>+</sup> significantly 15.53 mEq L <sup>-1</sup> to 8.57 mEq L <sup>-1</sup>	Ul Haq et al. (2016)
<i>Cucumis sativus</i> L.	MTW (40 mT)	Enhanced phosphorous content in leaves, lowered soil alkalinity,	Noran et al. (1970);
<i>Cucumis melo</i> L.	MF (40 mT)	enhanced nutrient uptake, but lowered the [Na <sup>+</sup> ] in the above ground plant parts	Shahin et al. (2016)



water treatment of seeds had been reported to improve the water productivity in the seedlings of eggplant (*Solanum melongena* L.), tomato (*Solanum lycopersicon* L.) and faba beans (*Vicia faba* L.) by 1.65, 1.70, and 1.88 times, and thereby, the water savings of 11%, 14.2% and 13.5% respectively (Kareem, 2018).

Exposure to a magnetic field causes several changes at the intracellular level like the density of  $\text{Ca}^{2+}$  and other ionic substances. The  $\text{Ca}^{2+}$  ions play a very important role in several aspects of plant responses-like its growth, photosynthetic activity, mineral uptake, water, and reserve food transportation. The exposure of sunflower (*Helianthus annuus*) seedlings to a vertical magnetic field of 0.02 mT strength promoted seedling growth parameters like root and shoot fresh weights (Fischer et al., 2004). Pre-sowing magnetic field exposures also resulted in the enlargement of epicotyl cells and the development of greater osmotic pressure in the seedlings. Further, low MF exposure causes the development of some ultrastructural peculiarities like higher buildup of lipid bodies, increased formation of different lytic compartments viz., vacuoles, cytosegresomes, and paramural bodies, and a subsequent decrease in the phyto-ferritin content in plastids. On the other hand, the mitochondria show most sensitivities such as the size and amount of mitochondria in cells is increased, the matrix of mitochondria get electro-transparent and reduction in cristae under exposure to low MF. Further, it has been reported that low MF exposure results in disruption of different metabolic systems with the  $\text{Ca}^{2+}$  homeostasis and this, in turn, leads to change in root cell ultrastructure (Belyavskaya, 2001).

Artificial shielding of geomagnetic field results in a substantial reduction in the cell numbers accompanied by higher DNA content in the meristematic region of shoot and root of onion (*Allium cepa*). Further, the formation of giant cells with large nuclei due to the fusion of normal nuclei, and binuclear or tetranuclear cells from mitosis without undergoing cytokinesis have been reported under low MF exposure (Nanushyan & Murashov, 2001). A recent study on the combination of magneto-priming and chemical priming on onion (*Allium cepa*) revealed that chemical priming with ascorbic acid (AsA) ( $50 \text{ mg L}^{-1}$ ) or zinc sulfate ( $500 \text{ mg L}^{-1}$ ) and 450 gauss of magneto-priming resulted in enhancement of growth parameters when the priming agents were used otherwise. The combination of AsA and magneto-priming for 24 hours also enhanced the germination velocity, dry weight, and vigor index of seedlings with reduced mean germination time, increased field emergence or total amino acids. Likewise, the combination of zinc sulfate and magneto-priming

increased the speed of field emergence and the seedling length (Zalama & Fathalla, 2020).

Seed treatment with MF of 0 to 250 mT with a step of 50 mT for 1 to 4 hours leads to enhance the growth parameters of chickpea root such as length, volume, and surface area of roots (Vashisth & Nagarajan, 2008). Vashisth et al. (2021) carried out an investigation on sunflower when the seeds were exposed to SMF of 200 mT for 2 hours prior to planting under soil moisture stress. They observed that there was remarkable enhancement in shoot length, leaf area index, leaf number, total biomass, seed's test weight with a higher seed yield containing more protein and oil. Interestingly an enhancement in terms of blue light-dependent anthocyanin build up or destruction of CRY2 (cryptochrome circadian regulator 2) protein has similarly been reported at greater intensities of MF (Ahmad et al., 2007). In another experiment, the seeds of three varieties of broad beans were treated with 12.5 nT of pulsed EMF with a frequency of 3 Hz for different time periods. Though 15 minutes of exposure resulted in enhanced vegetative parameters like the dry weight of the seedlings, chlorophyll contents, increased leaf area with higher transpiration rate and stomatal conductance but seed yield did not show any significant improvement as compared to control (Katsenios et al., 2020).

### **5.2.3 EFFECTS ON PHOTOSYNTHESIS AND CROP PRODUCTIVITY**

The chlorophyll content, stomatal conductance, and photosynthesis rate in leaves of maize (*Zea mays*) are increased upon treatment of seeds with a static MF of 100 and 200 mT (Anand et al., 2012). Further, electromagnetic priming of maize seeds at 100 and 150 mT for 10 minutes improves Chl-a content as well as photochemical and non-photochemical quenching, and subsequently, these changes lead to reduce the drought-induced adverse effect on plant growth (Javed et al., 2011). In another study, it has been reported that MF treatment on soybean seeds results in higher chlorophyll-a fluorescence, more soluble protein content in leaves with higher intensities of protein bands particularly in the 53 KDa larger subunit and 14 KDa smaller subunit of Rubisco along with a higher accumulation of biomass (Shine et al., 2011). However, contrasting results of reduced cotyledon area and dry weight along with decreased CO<sub>2</sub> uptake by the radish seedlings have also been observed when seeds are exposed to MF prior to sowing (Yano et al., 2004). MF exposure of 4 mT regardless of the direction promotes growth and CO<sub>2</sub> uptake by potato plantlets (Iimoto et al., 1996). The exposure of

seeds to a static MF of 50 mT for 2 h helps to increase root growth, leaf chlorophyll content, photosynthesis rate and various yield attributing traits like number of the effective tillers, number of grains per plant and test weight of grain which in turn result in higher grain production of wheat, and also help the plants to alleviate the adverse effect of salinity stress (Rathod & Anand, 2016). Magneto-priming of chickpea (*Cicer arietinum*) seeds has been found to increase yield by 33% and also to effectively mitigate the negative influences of salt stress in this crop (Thomas et al., 2013). Bilalis et al. (2012) have reported that pulsed EMFs exposure on maize seeds for 30 and 45 mins enhance the quantity of yields. A significant increase in the fruit yield of strawberries (*Fragaria vesca*) had also been described when strawberry plantlets were treated with MF in the range of 0.096 to 0.192 mT (Eşitken & Turan, 2004). Further, seed treatment with MF of 100 mT for 10 mins and 170 mT for 3 min has been observed to effectively increase the growth and yielding parameters of tomato like weight fruit<sup>-1</sup>, fruit plant<sup>-1</sup>, fruit yield area<sup>-1</sup> or fruit radius (De Souza et al., 2006).

#### **5.2.4 EFFECTS ON ALLEVIATION OF STRESS**

Abiotic stresses or environmental extremities such as salinity, water stress, high or low temperature, UV-B, etc., are well known to decrease germination rate, seedling vigor, nodulation, carbon, and nitrogen metabolism, growth rate, biomass production and ultimately crop yield. Researchers are of the opinion that the activation of pre-germinative metabolism as induced by the priming method would instill a kind of “stress memory” within seeds (Chen et al., 2012). As a consequence, such “stress memory” activates various protective mechanisms within the plant if it faces any stressful conditions during later stages of growth. Later on, the activated protective systems help the plant to alleviate the stress effects or also make the seedlings more tolerant to the specific kind of stress.

Pre-treatment of maize seeds with SMF 100 mT for 2 hours and 200 mT for 1 hour has been reported to help the plants to maintain the normal growth, water status of leaf and photosynthetic rate during the water stress. It is also noteworthy to mention that MF treatment shows reduced levels of free radicals and antioxidant enzyme activities in the leaves of 45-days old plants under mild and extreme water stress conditions in comparison to control plants (Anand et al., 2012). Seed priming with physical agents like static magnetic field has been reported to deliver compensating performance in respect of crop growth and yielding parameters both under normal and

abiotic stresses (Kataria & Jain, 2019). Further, the exposure of seeds to SMF of 200 mT for 1-hour has been reported to induce various positive responses such as the increasing activities of  $\alpha$ -amylase and protease, nitrate reductase, nitric oxide contents along with a reduction in hydrogen peroxide ( $H_2O_2$ ), superoxide ( $O_2^{\cdot-}$ ) and malondialdehyde (MDA) contents in leaves of soybean under UV-stress (Kataria et al., 2020). Moreover, magnetic field exposure on soybean has been reported to enhance the sugar and protein contents that would act as osmoprotectants in salt-affected callus and thereby minimize the adversities of salt stress (Radhakrishnan et al., 2012). Magnetopriming also reduces the impact of salt and drought stress in plants by enhancing the water uptake through root cells (Alvarez et al., 2020, 2021; Conrath, 2009; Kaya et al., 2006; Sarraf et al., 2020). It has been reported that an increase in  $Ca^{2+}$  ion would play a vital role in the drought tolerance mechanism under magnetic field exposure since the impairment between the cell's plasma membrane and photosynthetic apparatus is prevented by the enhanced  $Ca^{2+}$  and regulation of the phytohormone metabolisms in drought-affected plants (Blum, 1993; Selim & El-Nady, 2011; Song et al., 2008). Further, the enhanced production of proline and  $GA_3$  in MF treated plants would trigger the  $Mg^{2+}$  accumulation that results in the synthesis of chlorophyll and/or  $K^+$  accumulation that is responsible for an increased number of chloroplasts under drought stress (Reina & Pascual, 2001; Shaddad, 1990). Such enhancement of chlorophyll and chloroplast number in MF treated plants would then lead to developing thicker mesophyll tissues (Selim & El-Nady, 2011). In addition to these adaptive responses as induced by MF exposure, it also enhances stomatal conductance, increases  $CO_2$  concentration in sub-stomatal region of the leaves, or associated photochemical and non-photochemical reactions that help to improve drought tolerance of plants (Javed et al., 2011). The magnetic field exposure also positively influences the activities of several enzymes of polyamine pathways like ODC and PAL that help to withstand the adverse effects of biotic stresses or protect the plants during infection of plants by pathogens (Trebbi et al., 2007). A list of morpho-physiological or biochemical responses of plant species as triggered by magnetopriming under different abiotic stresses is summarized in Table 5.2. Apart from the above effects, the magnetic field exposure would also elicit several other changes such as a mutation in cells of stamen hair of the inflorescence, formation of longer pollen tubes with a faster germination rate, along with enhanced cell wall metabolism and changes in the orientation of the macromolecules (Alexander & Ganeshan, 1990; Baum & Nauman, 1984; Paul et al., 2006).

**TABLE 5.2** Magneto-Priming in the Alleviation of Various Stresses

Stress	Plants	Doses of Magneto-Priming Used to Alleviate Stress	Responses	References
Salinity stress	<i>Zea mays</i> L.	200 mT for 1 hour	Increased rate of seed germination, seedling growth, hydrolytic enzyme activities, efficiency of PSII, net photosynthetic rate, overall plant growth or yield along with a higher accumulation of free radicals	Baghel et al. (2019); Kataria et al. (2017b)
	<i>Cicer arietinum</i> L.	100 mT for 1 hour	Improved seed germination rate, root, and shoot growth, enhanced activity of $\alpha$ -amylase or protease as well as water uptake by roots	Thomas et al. (2013)
	<i>Glycine max</i> (Linn.) Merr.	200 mT for 1 hour	Increased number of root nodules, the content of leghemoglobin and hemichrome, plant biomass, crop yield, synthesis of photosynthetic pigments. Enhanced rate of photosynthesis, stomatal conductance, rate of transpiration and internal CO <sub>2</sub> concentration	Baghel et al. (2016)
	<i>Glycine max</i> (Linn.) Merr.	200 mT for 1 hour	Increased activities of $\alpha$ -amylase, protease, or nitrate reductase along with the accumulation of O <sub>2</sub> <sup>-</sup> , H <sub>2</sub> O <sub>2</sub> and NO	Kataria et al. (2020)
	<i>Triticum aestivum</i> L. and <i>Phaseolus vulgaris</i> L.	4 mT–7 mT SMF	Enhanced rate of seedling emergence, plant biomass production and growth	Cakmak et al. (2010)
	<i>Glycine max</i> L.	1,500 nT PMF @ 0.1, 1.0, 10.0, and 100.0 Hz	Improved root and shoot regeneration, biomass of callus, length, and number of roots, increased carbohydrates, proteins, secondary metabolites like flavonols, alkaloids or saponins contents	Radhakrishnan & Kumari (2013); Radhakrishnan et al. (2012)

**TABLE 5.2** (Continued)

Stress	Plants	Doses of Magneto-Priming Used to Alleviate Stress	Responses	References
Drought or water stress	<i>Glycine max</i> (Linn.) Merr.	200 mT SMF	Enhanced plant growth, synthesis of photosynthetic pigments, PSII efficiency, rate of photosynthesis and biomass production	Baghel et al. (2018)
	<i>Zea mays</i> L.	100 mT–150 mT EMF	Improved plant growth, chlorophyll content, rate of photosynthesis, transpiration rate, stomatal conductance, sub-stomatal CO <sub>2</sub> concentration, photochemical, and non-photochemical quenching reactions	Javed et al. (2011)
	<i>Zea mays</i> L.	100 mT–200 mT SMF	Enhanced plant growth, leaf water content and potential, stomatal turgor potentiality, photosynthesis, and stomatal conductance, but decreased activities of POX, CAT, and SOD	Anand et al. (2012)
	<i>Triticum aestivum</i> L.	2.9 mT–4.7 mT SMF	Increased chlorophyll and carotenoids contents but reduced activities of SOD, POX, APX, and CAT	Sen & Alikamanoglu (2014)
UV-B stress	<i>Glycine max</i> (Linn.) Merr.	200 mT SMF	Increased plant growth, PSII or photosynthetic efficiency, crop yield, activities of various enzymes like carbonic anhydrase or nitrogenase, nucleic acids, protein, and NO contents in leaves, decreased levels of ROS with improved antioxidant defense mechanisms	Kataria et al. (2017a, 2020)
Temperature and light stress	<i>Zea mays</i> L.	150 mT SMF	Enhanced chilling tolerance, increased plant growth, chlorophyll, and total phenolic contents, improved gas exchange parameters, increased protein, or oil contents in seed but reduced membrane permeability	Afzal et al. (2015)
	<i>Raphanus sativus</i> L.	400 A/m WMF	Increased levels of glycolipids, phospholipids as well as phospholipids to sterols ratio	Novitskaya et al. (2010)

**TABLE 5.2** (Continued)

Stress	Plants	Doses of Magneto-Priming Used to Alleviate Stress	Responses	References
Heavy metal stress (Cadmium stress)	<i>Vigna radiata</i> L.	600 mT	Improved plant growth, photosynthesis rate, nitric oxide synthase as well as NO content but reduced membrane permeability, electrolyte leakage, H <sub>2</sub> O <sub>2</sub> and O <sub>2</sub> <sup>-</sup> contents	Chen et al. (2011)
Biotic stress	<i>Citrus aurantifolia</i> L.	10 kHz WMF	Enhanced leaf biomass, proline, and protein content, increased MDA accumulation but reduced H <sub>2</sub> O <sub>2</sub> and carbohydrates contents	Abdollahi et al. (2012)
	<i>Nicotiana tabacum</i> L.	-17 to 13 $\mu$ T SMF and 25.6 to 28.9 $\mu$ T SSMF @ 10 Hz	Improved activities of enzymes like ODC or PAL but reduced the number and area of lesions	Trebbei et al. (2007)

### 5.3 UNDERLYING MECHANISMS OF MAGNETO-PRIMING

Various biochemical traits remain on the basis of the regulation of plant growth and development. The magnetic field would possibly alter these biochemical processes such as activities of several enzymes, ion flux across the plasma membrane, production of secondary metabolites or growth regulators and their transportations, etc., and thereby it would play an essential function in the regulation of plant growth and development (Leelapriya et al., 2003). It is thought that a combination of range, pitch, and higher frequency of non-uniform magnetic field would generate the so-called 'ponderomotive force' which subsequently affects biological systems (Balcavage et al., 1996; De Souza et al., 2006). It is assumed that positive influences of the magnetic field resulting from this 'ponderomotive force' that triggers auxin action and subsequently it results in increasing cell wall extensibility, altered ion transport across the cell membrane, or the regulation of a varied set of developmental processes through alterations in cell division, cell elongation, cell differentiation, as well as cell or organ polarity. Another thought indicates that an increase in the activities of certain enzymes would be the reason for biological effects under magnetic treatments. Since the Earth's geomagnetic field can alter the activities of important enzymes like  $\text{Ca}^{2+}$ /CaM-dependent PDEs (Cyclic nucleotide phosphodiesterases) that play an important role in between the cyclic nucleotides and  $\text{Ca}^{2+}$  mediated second messenger system. Further, it would also change the activity of cytochrome C oxidase, a key enzyme that participates in ATP synthesis through the redox reactions in mitochondria or initiates program cell death on receiving apoptotic stimulus (Liboff et al., 2003; Nossol et al., 1993). It has also been evident that a magnetic field of 0.2 and 0.3  $\mu\text{T}$  can positively stimulate  $\text{Na}^+$  and  $\text{K}^+$ -ATPase activity (Blank & Soo, 1996). Further, the magnetic field of 30 mT causes enhancement of the esterase activity in wheat and MF 1 mT increases peroxidase activity in horseradish (Aksenov et al., 2000; Portaccio et al., 2005).

Alteration in the cell membrane ultrastructure leading to increased cellular permeability enhanced water flow through vascular tissues and transportation of ions through ion channels are supposed to be important behind the changes in the metabolic pathways under magnetic exposure (Bondarenko et al., 1996; Jamil & Ahmad, 2012). The increase in cellular permeability would then result in rapid imbibition of water and subsequently, it leads to *de novo* synthesis of hydrolytic enzymes. Hydrolytic enzymes like  $\alpha$ -amylase are an endohydrolase that liberates smaller glucans by cleaving the  $\alpha$ -1, 4-linkages between glucosyl residues. On the other hand, protease



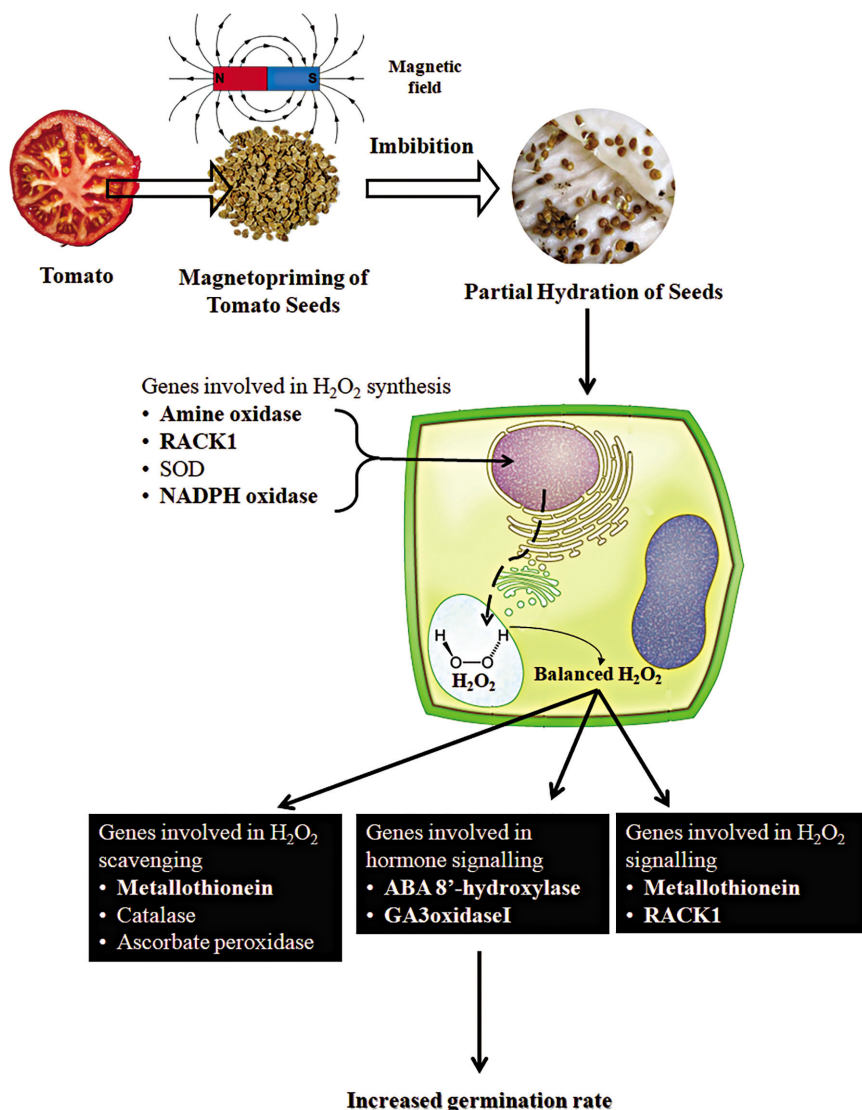
is an important enzyme that plays an active role in amino acid production in germinating seeds. The water-insoluble storage proteins are hydrolyzed by endo-proteases and converted into soluble peptides or finally into amino acids. The higher germination rate under magnetic field exposures would be correlated with the greater mobilization of reserve food materials due to greater activity of enzymes namely,  $\alpha$ -amylase, protease, and dehydrogenase in germinating seeds of several crops (Bhardwaj et al., 2012; Bhatnagar & Deb, 1978; Samani et al., 2013). Though contradictory results regarding the negative influence of MF on the protease activity on the second and fourth day of germination in broad beans have also been recorded (Rajendra et al., 2005). MF treatment has been reported to enhance the activity of cambium differentiation that leads to the formation of a higher amount of xylem and phloem tissues, and thereby it improves the water and nutrient absorption capacity or their transportation through the plant system that ultimately results in improved plant health (Selim & El-Nady, 2011).

It is also assumed that capability of absorbing magnetic energy by cellular ions is important for magnetic influence on biological systems. Further, the ionic changes or their motions and the physiological availability of small ions in the cell as affected by the specific frequencies and strength of EMFs would be responsible for the particular biological effects on the plants (Pazur & Rassadina, 2009). Previously it has been proposed that the interactions between magnetic field and biological system would involve several possible mechanisms viz.: (i) cyclotron resonance model; (ii) paramagnetic resonance model; (iii) interference between the ions and electron at quantum state; (iv) free radical reactions including other spin mechanisms; (v) compatible quantum incitement; (vi) biological effects of torsion fields associated with weak magnetic field; (vii) oscillator models both at classical and quantum state; (viii) active metastable state of liquid water by biological systems; (ix) amplifying mechanism through stochastic resonance or other indiscriminate processes; and (x) phase conversions revealing liquid crystal assembly in biophysical systems, etc. (Maffei, 2014). However, according to the recent insights, there are three main types of magneto sensing mechanism, i.e.: (a) the radical pair mechanism which is an interaction between the magnetic nucleus and the unpaired electrons of the radicals that results in the breaking of the symmetry of two electronic spins or the interconversion of the spins either in antiparallel direction/singlet state or parallel direction/triplet state; (b) the cyclotron resonance model of ions suggesting that the Larmor frequencies of the charged particles like ions follow a circular motion in a perpendicular plane to the external magnetic field; and (c) cryptochromes,

the blue light-absorbing flavoproteins are assumed to help several organisms like birds, insects or plants in perceiving the direction and intensities of the magnetic field. Upon exposure to blue light, these flavoproteins undergo a redox cycle of flavins and tryptophan residue that leads to the activation of radical pair mechanism and this, in turn, results in the perception of magnetic field (Fay et al., 2019; Mridha et al., 2016; Pooam et al., 2019). Another possibility “ferrimagnetism hypothesis” indicates that perception of static magnetic fields is accomplished through its interaction with iron particles, inorganic iron compounds like iron oxide black or greigite as well as with phytoferritin or iron-containing proteins of the plant cells.

Though it is well known that enhanced production of cellular ROS would negatively affect the functioning of plant systems, but the magnetic treatment causes to reduce ROS production and improve plant growth significantly under different stresses (Radhakrishnan, 2019). In germinating seeds, ROS are generated via aerobic metabolisms that occur in mitochondria, chloroplasts, peroxisomes, and/or the apoplastic spaces of the cell membrane. The production of ROS is initiated by the active participation of some enzymes like xanthine oxidase (XO), superoxide dismutase (SOD), lipoxygenases (LOXs), membrane bound NADPH oxidase (NOX), amine oxidases (AOs) or peroxidases (POXs), etc. The reaction chain can be described as there is a redox-active metal ion present in each enzyme, and the metal ion initiates the generation of superoxide molecules, and then reduction of superoxide by the metal ion yields hydrogen peroxide ( $H_2O_2$ ) (Bolwell & Wojtaszek, 1997; Collin, 2019; Messner & Imlay, 2002; Munro & Treberg, 2017; Smirnov & Arnaud, 2019). These generated ROS play an important role as signaling molecules for the major events that happened in germinating seeds including dormancy breaking. The crucial roles of ROS like  $H_2O_2$ , hydroxyl radical ( $\bullet OH$ ), superoxide ( $O_2^{\bullet -}$ ) in seed germination of different crops have been established by several research (Bhardwaj et al., 2012; Kranner et al., 2010; Morohashi, 2002; Müller et al., 2009; Schopfer, 2001).  $H_2O_2$  is the most common among the ROS. It stays for a long time in the plant systems, and it can easily diffuse through the cell membrane to reach their action sites from the actual sites of production. Actually, the redox state of the seed is changed by the interaction between ROS and the hormones that participate in seed germination. The participation of these hormones determines the success or failure of germination through the initiation of the metabolic reactions by regulating the expression of related genes (Bailly et al., 2008; El-Maarouf-Bouteau & Bailly, 2008). In this context, it is also thought that there is an antagonistic effect between gibberellic acid (GA) and abscisic acid (ABA)

during the germination events (Koornneef et al., 2002). GA upregulates the process of germination and dormancy breakdown whereas ABA downregulates the process and causes the continuation of the dormancy period. Several changes in plant systems with and without magnetic influence are presented schematically in Figure 5.1.



**FIGURE 5.1** Possible changes in plant systems with and without magnetic field exposure.

However, the magnetic field exposure has also been reported to increase the production and activity of several ROS scavenging enzymes. There are two types of antioxidant system viz., an enzymatic system which is mediated by enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPOX), glutathione reductase (GR), glutathione s-transferase (GST), etc., and non-enzymatic system comprised of different phenolic compounds, carotenoids, AsA,  $\alpha$ -tocopherol, etc. The antioxidant systems help to minimize the uncontrolled oxidation and thereby protect the plant from oxidative damages induced by ROS (Foyer & Noctor, 2005). The ROS scavenging enzymes such as SOD, CAT, APX, etc., regulate the concentration of ROS within the cell. Actually, the antioxidant systems maintain a critical level of ROS concentration that is called “oxidative window” and thus allow the ROS to work as signaling molecules (Figure 5.2). The progressive events of germination are not permitted if the concentration of ROS is below or above the “oxidative window” (Bailly et al., 2008). Recent findings of the higher transcript level of some genes that are involved in the synthesis of SOD1, SOD9, NOX, and AO in germinating seeds under MF exposure would indicate the crucial role of magnetopriming in ROS scavenging (Anand et al., 2019).

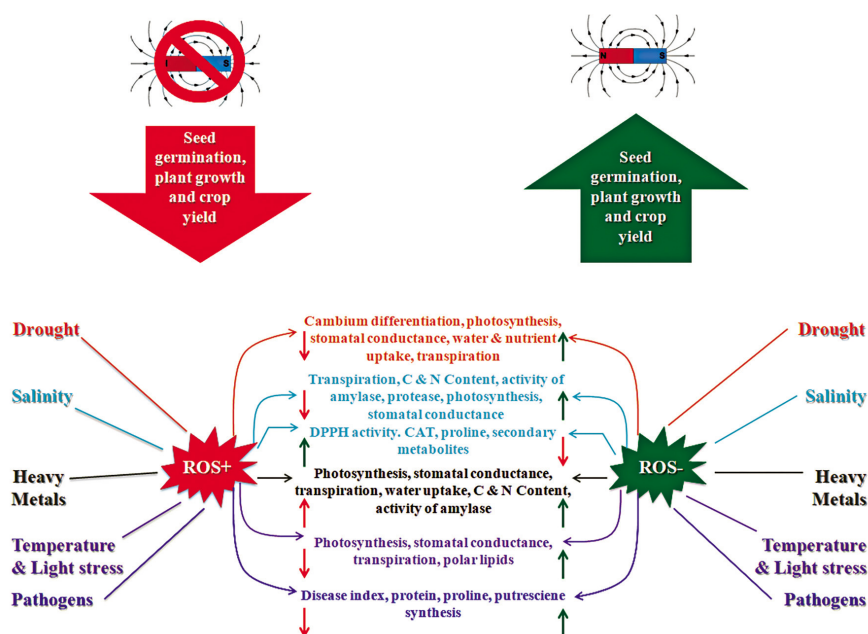


FIGURE 5.2 MF induced activation of antioxidant systems in germinating seeds.

## **5.4 ADVANTAGES AND DISADVANTAGES OF MAGNETO-PRIMING**

As like every technology, seed pre-magnetization also delivers both advantages and disadvantages. The advantages of magneto-priming would be summarized as:

- This is a simple, cost-effective, and harmless scientific technique of seed invigoration.
- The magnetic field can be of various types like a geomagnetic field, static magnetic field, the EMF of AC (alternating current) and DC (direct current), magnetically treated water, etc., and the strengths of the magnetic field can easily be regulated. Due to such versatility in experimental designs, the magnetic field can induce different responses in the plant system.
- The use of magneto-priming is not only restricted to laboratory experiments rather can also be extended to field applications since pre-sowing exposure of seeds to the magnetic field has been described to have positive influences on plant growth parameters like plant height, leaf area expansion, leaf chlorophyll content, photosynthesis rate and plant biomass production.
- Pre-sowing magnetization of seeds is able to mitigate several stress conditions like salinity, drought, UV-B exposure, heavy metals like cadmium as well as biotic stresses. Thus, magneto-priming is one the most effective priming technologies to increase a plant's survival and productivity under environmental stress conditions too.
- The use of physical priming techniques like magneto-priming can be an effective and eco-friendly approach in reducing the use of hazardous chemicals in agriculture to control pests and diseases.

Despite having the above advantages, magneto-priming has some disadvantages too:

- The main disadvantage of magneto-priming is the lack of proper information about the interactions of MF at the molecular and genetic levels.
- Besides, there is a lack of protocol for the experimental designs and standardized crop-specific intensities of the magnetic field. Further, the use of high strength EMFs can be hazardous to human health and thus it requires proper expertise on how to construct and sustain the experimental design.

## **5.5 CONCLUSION**

Our planet Earth has its own magnetic field that is known as the geomagnetic field and this geomagnetic field has obvious consequences on the biosphere present on the Earth. Further, with the advent of the progress of science during the last century and with the events of modernization of our society, we are now habituated to using many types of equipment in our daily life ranging from scientific appliances to medical instruments or from mobile towers to latest war devices, and thereby we are being exposed knowingly or inadvertently to the magnetic field at every moment. Therefore, these man-made magnetic fields of various kinds and strengths along with the geomagnetic field are constantly influencing the living beings on the Earth including the crop plants. Although the magnetic field of higher strength has some negative influences on plants depending on the types of species and the environmental conditions, but many researchers have already established that magnetic field at low intensity can improve overall growth and development of plants, crop productivity, and adaptations against different stresses. Thus, the magnetic exposure of seeds prior to sowing would be an effective modulator in the agricultural sector to build a good crop stand, improve plant growth and enhance yield. However, the elucidation of the effect of geophysical fields such as the geomagnetic field on plants is very challenging as the setting up of specific experimental design is not only problematic rather hard to comprehend. Till now, we have very little information about the biochemical reactions, physiological processes, and expression of specific genes or molecular mechanisms happening within living organisms after being exposed to a magnetic field and this indicates that a lot of knowledge is yet to be accomplished in this field. Thus, there is an urgent need to undertake extensive research on the influences of magnetic fields on crop plants as well as on the other living organisms present on the Earth to unravel the mechanisms involved in seed magnetization completely. Such research would lead to explore various pros and cons of seed magnetization and to recommend this practice as a cost-effective and environment-friendly agro-technique towards food security and agricultural sustainability.

## KEYWORDS

- **abiotic stress**
- **cost-effective method**
- **crop growth**
- **electromagnetic field**
- **magnetized water**
- **magneto-priming**
- **plant responses**
- **static magnetic field**

## REFERENCES

- Abdollahi, F., Niknam, V., Ghanati, F., Masroor, F., & Noorbakhsh, S. N., (2012). Biological effects of weak electromagnetic field on healthy and infected lime (*Citrus aurantifolia*) trees with phytoplasma. *Sci. World J.*, 2012.
- Abdolmaleki, P., Ghanati, F., Sahebamei, H., & Sarvestani, A. S., (2007). Peroxidase activity, lignification and promotion of cell death in tobacco cells exposed to static magnetic field. *The Environmentalist*, 27(4), 435–440.
- Afzal, I., Mukhtar, K., Qasim, M., Basra, S., Shahid, M., & Haq, Z., (2012). Magnetic stimulation of marigold seed. *Int. Agrophys.*, 26(4), 335–339.
- Afzal, I., Noor, M., Bakhtavar, M., Ahmad, A., & Haq, Z., (2015). Improvement of spring maize performance through physical and physiological seed enhancements. *Seed Sci. Technol.*, 43(2), 238–249.
- Ahamed, M., Elzaawely, A., & Bayoumi, Y., (2013). Effect of magnetic field on seed germination, growth and yield of sweet pepper (*Capsicum annuum* L.). *Asian J. Crop Sci.*, 5(3), 286–294.
- Ahmad, M., Galland, P., Ritz, T., Wiltshko, R., & Wiltshko, W., (2007). Magnetic intensity affects cryptochrome-dependent responses in *Arabidopsis thaliana*. *Planta.*, 225(3), 615–624.
- Aksenov, S., Bulychev, A., Tlu, G., & Turovetskiĭ, V., (2000). Effect of a low-frequency magnetic field on esterase activity and change in pH in wheat germ during swelling of wheat seeds. *Biofizika.*, 45(4), 737–745.
- Aksyonov, S., Grunina, T. Y., & Goryachev, S., (2007). On the mechanisms of stimulation and inhibition of wheat seed germination by low-frequency magnetic field. *Biophys.*, 52(2), 233–236.
- Aladjadjiyan, A., (2002). Study of the influence of magnetic field on some biological characteristics of *Zea mays*. *J. Cent. Eur. Agric.*, 3(2), 89–94.
- Aladjadjiyan, A., (2007). The use of physical methods for plant growing stimulation in Bulgaria. *J. Cent. Eur. Agric.*, 8(3), 369–380.

- Alemán, E. I., Mboghli, A., Boix, Y. F., González-Olmedo, J., & Chalfun-Junior, A., (2014). Effects of EMFs on some biological parameters in coffee plants (*Coffea arabica* L.) obtained by *in-vitro* propagation. *Pol. J. Environ. Stud.*, 23(1), 95–101.
- Alexander, M., & Ganeshan, S., (1990). Electromagnetic field induced *in-vitro* pollen germination and tube growth. *Curr. Sci.*, 59(5), 276–277.
- Alvarez, J., Martinez, E., Carbonell, V., & Florez, M., (2020). Effects of polyethylene glycol and sodium chloride stress on water absorption of magneto-primed triticale seeds. *Rom. Rep. Phys.*, 72, 708.
- Alvarez, J., Martinez, E., Florez, M., & Carbonell, V., (2021). Germination performance and hydro-time model for magneto-primed and osmotic-stressed triticale seeds. *Rom. J. Phys.*, 66, 801.
- Anand, A., Kumari, A., Thakur, M., & Koul, A., (2019). Hydrogen peroxide signaling integrates with phytohormones during the germination of magnetoprimed tomato seeds. *Sci. Rep.*, 9(1), 1–11.
- Anand, A., Nagarajan, S., Verma, A., Joshi, D., Pathak, P., & Bhardwaj, J., (2012). Pre-treatment of seeds with static magnetic field ameliorates soil water stress in seedlings of maize (*Zea mays* L.). *Indian J. Biochem. Biophys.*, 49(1), 63–70.
- Athari, N. M., Noori, M., & Ghanati, F., (2008). Effect of static magnetic field on certain physiological and biochemical features of *Cicer arietinum* in vegetative growth phase. *Pajouhesh-Va-Sazandegi.*, 21(3), 62–68.
- Azimi, N., Majd, A., Nejadstattari, T., Ghanati, F., & Arbabian, S., (2018). Effects of magnetically treated water on physiological characteristics of *Lens culinaris* L. *Iran. J. Sci. Technol. Trans. A Sci.*, 42(2), 331–337.
- Baghel, L., Kataria, S., & Guruprasad, K. N., (2016). Static magnetic field treatment of seeds improves carbon and nitrogen metabolism under salinity stress in soybean. *Bio Electromagn.*, 37(7), 455–470.
- Baghel, L., Kataria, S., & Guruprasad, K., (2018). Effect of static magnetic field pretreatment on growth, photosynthetic performance and yield of soybean under water stress. *Photosynthetica.*, 56(2), 718–730.
- Baghel, L., Kataria, S., & Jain, M., (2019). Mitigation of adverse effects of salt stress on germination, growth, photosynthetic efficiency and yield in maize (*Zea mays* L.) through magnetopriming. *Acta Agrobot.*, 72(1).
- Bailly, C., El-Maarouf-Bouteau, H., & Corbineau, F., (2008). From intracellular signaling networks to cell death: The dual role of reactive oxygen species in seed physiology. *Comptes. Rendus. Biol.*, 331(10), 806–814.
- Balcavage, W., Alvager, T., Swez, J., Goff, C., Fox, M., Abdullyava, S., & King, M., (1996). A mechanism for action of extremely low frequency electromagnetic fields on biological systems. *Biochem. Biophys. Res. Commun.*, 222(2), 374–378.
- Baum, J. W., & Nauman, C. H., (1984). Influence of strong magnetic fields on genetic endpoints in *tradescantia* tetrads and stamen hairs. *Environ. Mutagen.*, 6(1), 49–58.
- Belov, K., & Bochkarev, N., (1983). *Magnetism on Earth and in Space*. Izdatel'stvo Nauka: Moscow.
- Belyavskaya, N., (2001). Ultrastructure and calcium balance in meristem cells of pea roots exposed to extremely low magnetic fields. *Adv. Space Res.*, 28(4), 645–650.
- Belyavskaya, N., (2004). Biological effects due to weak magnetic field on plants. *Adv. Space Res.*, 34(7), 1566–1574.



- Bhardwaj, J., Anand, A., & Nagarajan, S., (2012). Biochemical and biophysical changes associated with magnetopriming in germinating cucumber seeds. *Plant Physiol. Biochem.*, 57, 67–73.
- Bhatnagar, D., & Deb, A., (1978). Some effects of pre-germination exposure of wheat seeds to magnetic field. *Seed Res.*, 6, 1–14.
- Bilalis, D. J., Katsenios, N., Efthimiadou, A., & Karkanis, A., (2012). Pulsed electromagnetic field: An organic compatible method to promote plant growth and yield in two corn types. *Electromagn. Biol. Med.*, 31(4), 333–343.
- Blank, M., & Soo, L., (1996). The threshold for Na, K-ATPase stimulation by electromagnetic fields. *Bioelectrochem. Bioenerg.*, 40(1), 63–65.
- Blum, A., (1993). Selection for sustained production in water-deficit environments. *Int. Crop Sci. I.*, 343–347.
- Bogatina, N., Verkin, B., & Kordyum, V., (1978). Effect of permanent magnetic fields with different intensities on the wheat growth rate. *Dokl. Akad. Nauk Ukr. SSR Ser. B.*, 4, 352–356.
- Bolwell, G. P., & Wojtaszek, P., (1997). Mechanisms for the generation of reactive oxygen species in plant defense—a broad perspective. *Physiol. Mol. Plant Path.*, 51(6), 347–366.
- Bondarenko, N., Rokhinson, E., Gak, E., & Klygina, L., (1996). *Magnetic Equipment in Agriculture* (pp. 30–34). Russian agricultural sciences C/C of reports, Russian Academy of Agricultural Sciences.
- Braga, R. A., Azevedo, R. L. D., Guimarães, R. M., & Reis, L. V., (2020). Magnetic field in coffee seed germination. *Cienc. Agrotec.*, 44.
- Cakmak, T., Cakmak, Z. E., Dumlupinar, R., & Tekinay, T., (2012). Analysis of apoplastic and symplastic antioxidant system in shallot leaves: Impacts of weak static and electric magnetic field. *J. Plant Physiol.*, 169(11), 1066–1073.
- Cakmak, T., Dumlupinar, R., & Erdal, S., (2010). Acceleration of germination and early growth of wheat and bean seedlings grown under various magnetic field and osmotic conditions. *Bio Electromagn.*, 31(2), 120–129.
- Chen, K., Fessehaie, A., & Arora, R., (2012). Dehydrin metabolism is altered during seed osmopriming and subsequent germination under chilling and desiccation in *Spinacia oleracea* L. cv. Bloomsdale: Possible role in stress tolerance. *Plant Sci.*, 183, 27–36.
- Chen, Y. P., Li, R., & He, J. M., (2011). magnetic field can alleviate toxicological effect induced by cadmium in mungbean seedlings. *Ecotoxicol.*, 20(4), 760–769.
- Collin, F., (2019). Chemical basis of reactive oxygen species reactivity and involvement in neurodegenerative diseases. *Int. J. Mol. Sci.*, 20(10), 2407.
- Conrath, U., (2009). Priming of induced plant defense responses. *Adv. Bot. Res.*, 51, 361–395.
- Da Silva, J. A. T., & Dobránszki, J., (2016). Magnetic fields: How is plant growth and development impacted? *Protoplasma.*, 253(2), 231–248.
- De Souza, A., Garcí, D., Sueiro, L., Gilart, F., Porras, E., & Licea, L., (2006). Pre-sowing magnetic treatments of tomato seeds increase the growth and yield of plants. *Bio Electromagn.*, 27(4), 247–257.
- De Souza, A., Sueiro, L., García, D., & Porras, E., (2010). Extremely low frequency non-uniform magnetic fields improve tomato seed germination and early seedling growth. *Seed Sci. Technol.*, 38(1), 61–72.
- Dhawi, F., (2014). Why magnetic fields are used to enhance a plant's growth and productivity? *Annu. Res. Rev. Biol.*, 4(6), 886–896.

- Eftimiadou, A., Katsenios, N., Karkanis, A., Papastylianou, P., Triantafyllidis, V., Travlos, I., & Bilalis, D. J., (2014). Effects of presowing pulsed electromagnetic treatment of tomato seed on growth, yield, and lycopene content. *Sci. World J.*, 2014, 369745.
- El-Maarouf-Bouteau, H., & Bailly, C., (2008). Oxidative signaling in seed germination and dormancy. *Plant Signal Behav.*, 3(3), 175–182.
- Eşitken, A., & Turan, M., (2004). Alternating magnetic field effects on yield and plant nutrient element composition of strawberry (*Fragaria x ananassa* cv. *Camarosa*). *Acta Agric. Scand. B Soil Plant Sci.*, 54(3), 135–139.
- Fay, T. P., Lindoy, L. P., Manolopoulos, D. E., & Hore, P., (2019). How quantum is radical pair magnetoreception? *Faraday Discuss.*, 221, 77–91.
- Fischer, G., Tausz, M., Köck, M., & Grill, D., (2004). Effects of weak 16½ Hz magnetic fields on growth parameters of young sunflower and wheat seedlings. *Bio Electromagn.*, 25(8), 638–641.
- Florez, M., Alvarez, J., Martinez, E., & Carbonell, V., (2019). Stationary magnetic field stimulates rice roots growth. *Rom. Rep. Phys.*, 71, 713.
- Foyer, C. H., & Noctor, G., (2005). Oxidant and antioxidant signaling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ.*, 28(8), 1056–1071.
- Haghighat, N., Abdolmaleki, P., Ghanati, F., Behmanesh, M., & Payez, A., (2014). Modification of catalase and MAPK in *Vicia faba* cultivated in soil with high natural radioactivity and treated with a static magnetic field. *J. Plant Physiol.*, 171(5), 99–103.
- Hasan, M. M., Alharby, H. F., Hajar, A. S., Hakeem, K. R., & Alzahrani, Y., (2019). The effect of magnetized water on the growth and physiological conditions of *Moringa spp.* under drought stress. *Pol. J. Environ. Stud.*, 8, 1145–1155.
- Hasenstein, K. H., John, S., Scherp, P., Povinelli, D., & Mopper, S., (2013). Analysis of magnetic gradients to study gravitropism. *Am. J. Bot.*, 100(1), 249–255.
- Hilal, M., Shata, S., Abdel-Dayem, A., & Hilal, M., (2002). Application of magnetic technologies in desert agriculture: III. effect of magnetized water on yield and uptake of certain elements by *citrus* in relation to nutrients mobilization in soil. *Egypt. J. Soil Sci.*, 42, 43–56.
- Hirota, N., Nakagawa, J., & Kitazawa, K., (1999). Effects of a magnetic field on the germination of plants. *J. Appl. Phys.*, 85(8), 5717–5719.
- Hozayn, M., EL-Mahdy, A., & Zalama, M., (2018). Magnetopriming for improving germination, seedling attributes and field performance of barley (*Hordeum vulgare* L.) under salinity stress. *Middle East J. Agric. Res.*, 7, 1006–1022.
- Iimoto, M., Watanabe, K., & Fujiwara, K., (1996). Effects of magnetic flux density and direction of the magnetic field on growth and CO<sub>2</sub> exchange rate of potato plantlets in vitro. In *International Symposium on Plant Production in Closed Ecosystems*, 440, 606–610.
- Ijaz, B., Jatoi, S. A., Ahmad, D., Masood, M. S., & Siddiqui, S. U., (2012). Changes in germination behavior of wheat seeds exposed to magnetic field and magnetically structured water. *Afr. J. Biotechnol.*, 11(15), 3575–3585.
- Jamil, Y., & Ahmad, M. R., (2012). Effect of pre-sowing magnetic field treatment to garden pea (*Pisum Sativum* L.) seed on germination and seedling growth. *Pak. J. Bot.*, 44, 1851–1856.
- Javed, N., Ashraf, M., Akram, N. A., & Al-Qurainy, F., (2011). Alleviation of adverse effects of drought stress on growth and some potential physiological attributes in maize (*Zea mays* L.) by seed electromagnetic treatment. *Photochem. Photobiol.*, 87(6), 1354–1362.

- Jouni, F. J., Abdolmaleki, P., & Ghanati, F., (2012). Oxidative stress in broad bean (*Vicia faba* L.) induced by static magnetic field under natural radioactivity. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.*, 741(1, 2), 116–121.
- Kareem, N. S. A., (2018). Evaluation of magnetizing irrigation water impacts on the enhancement of yield and water productivity for some crops. *J. Agric. Sci. Technol. A.*, 8, 271–283.
- Kataria, S., & Jain, M., (2019). Magnetopriming alleviates the adverse effects of abiotic stresses in plants. In: *Plant Tolerance to Environmental Stress* (pp. 427–442). CRC Press: United States.
- Kataria, S., Baghel, L., & Guruprasad, K., (2017). Alleviation of adverse effects of ambient UV stress on growth and some potential physiological attributes in soybean (*Glycine max*) by seed pre-treatment with static magnetic field. *J. Plant Growth Regul.*, 36(3), 550–565.
- Kataria, S., Baghel, L., & Guruprasad, K., (2017). Pre-treatment of seeds with static magnetic field improves germination and early growth characteristics under salt stress in maize and soybean. *Biocatal. Agric. Biotechnol.*, 10, 83–90.
- Kataria, S., Baghel, L., Jain, M., & Guruprasad, K., (2019). Magnetopriming regulates antioxidant defense system in soybean against salt stress. *Biocatal. Agric. Biotechnol.*, 18, 101090.
- Kataria, S., Jain, M., Tripathi, D. K., & Singh, V. P., (2020). Involvement of nitrate reductase-dependent nitric oxide production in magnetopriming-induced salt tolerance in soybean. *Physiol. Plant.*, 168(2), 422–436.
- Kataria, S., Rastogi, A., Bele, A., & Jain, M., (2020). Role of nitric oxide and reactive oxygen species in static magnetic field pre-treatment induced tolerance to ambient UV-B stress in soybean. *Physiol. Mol. Biol. Plants.*, 26(5), 931–945.
- Katsenios, N., Sparangis, P., Kakabouki, I., & Efthimiadou, A., (2020). Influence of pulsed electromagnetic field as a pre-sowing treatment on germination, plant growth and yield of broad beans. *Not. Bot. Horti Agrobot. Cluj-Na.*, 48(3), 1398–1412.
- Kaya, M. D., Okçu, G., Atak, M., Cıkılı, Y., & Kolsarıcı, Ö., (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Eur. J. Agron.*, 24(4), 291–295.
- Kobayashi, M., Soda, N., Miyo, T., & Ueda, Y., (2004). Effects of combined DC and AC magnetic fields on germination of hornwort seeds. *Bio Electromagn.*, 25(7), 552–559.
- Konefał-Janocha, M., Banaś-Ząbczyk, A., Bester, M., Bocak, D., Budzik, S., Górny, S., Larsen, S., et al., (2018). The effect of stationary and variable electromagnetic fields on the germination and early growth of radish (*Raphanus sativus*). *Pol. J. Environ. Stud.*, 28(2), 709–715.
- Koornneef, M., Bentsink, L., & Hilhorst, H., (2002). Seed dormancy and germination. *Curr. Opin. Plant Biol.*, 5(1), 33–36.
- Kordas, L., (2002). The effect of magnetic field on growth, development and the yield of spring wheat. *Pol. J. Environ. Stud.*, 11(5), 527–530.
- Kranner, I., Roach, T., Beckett, R. P., Whitaker, C., & Minibayeva, F. V., (2010). Extracellular production of reactive oxygen species during seed germination and early seedling growth in *Pisum sativum*. *J. Plant Physiol.*, 167(10), 805–811.
- Krylov, A., (1960). Magnetotropism of plants and its nature. *Fiziologiya Rastienji.*, 7, 917–919.

- Leelapriya, T., Dhilip, K., & Sanker, N. P., (2003). Effect of weak sinusoidal magnetic field on germination and yield of cotton (*Gossypium spp.*). *Electromagn. Biol. Med.*, 22(2, 3), 117–125.
- Liboff, A., Cherng, S., Jenrow, K., & Bull, A., (2003). Calmodulin-dependent cyclic nucleotide phosphodiesterase activity is altered by 20  $\mu$ T magnetostatic fields. *Bio Electromagn.*, 24(1), 32–38.
- Maffei, M. E., (2014). Magnetic field effects on plant growth, development, and evolution. *Front. Plant Sci.*, 5, 445.
- Mahajan, T. S., & Pandey, O. P., (2014). Magnetic-time model at off-season germination. *Int. Agrophys.*, 28(1), 57–62.
- Maheshwari, B. L., & Grewal, H. S., (2009). Magnetic treatment of irrigation water: Its effects on vegetable crop yield and water productivity. *Agric. Water Manag.*, 96(8), 1229–1236.
- Menegatti, R. D., De Oliveira, L. O., Da Costa, Á. V. L., Braga, E. J. B., & Bianchi, V. J., (2019). Magnetic field and gibberellic acid as pre-germination treatments of passion fruit seeds. *Rev. Ciênc. Agríc.*, 17(1), 15–22.
- Messner, K. R., & Imlay, J. A., (2002). Mechanism of superoxide and hydrogen peroxide formation by fumarate reductase, succinate dehydrogenase, and aspartate oxidase. *J. Biol. Chem.*, 277(45), 42563–42571.
- Mohammadi, R., & Roshandel, P., (2020). Ameliorative effects of a static magnetic field on hyssop (*Hyssopus officinalis* L.) growth and phytochemical traits under water stress. *Bio Electromagn.*, 41(6), 403–412.
- Morohashi, Y., (2002). Peroxidase activity develops in the micropylar endosperm of tomato seeds prior to radicle protrusion. *J. Exp. Bot.*, 53(374), 1643–1650.
- Mridha, N., Chattaraj, S., Chakraborty, D., Anand, A., Aggarwal, P., & Nagarajan, S., (2016). Pre-sowing static magnetic field treatment for improving water and radiation use efficiency in chickpea (*Cicer arietinum* L.) under soil moisture stress. *Bio Electromagn.*, 37(6), 400–408.
- Mroczek-Zdyrska, M., Tryniecki, Ł., Kornarzyński, K., Pietruszewski, S., & Gagoś, M., (2021). Influence of magnetic field stimulation on the growth and biochemical parameters in *Phaseolus vulgaris* L. *J. Microbiol. Biotechnol. Food Sci.*, 2021, 548–551.
- Müller, K., Linkies, A., Vreeburg, R. A., Fry, S. C., Krieger-Liszkay, A., & Leubner-Metzger, G., (2009). In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. *Plant Physiol.*, 150(4), 1855–1865.
- Munro, D., & Treberg, J. R., (2017). A radical shift in perspective: Mitochondria as regulators of reactive oxygen species. *J. Exp. Biol.*, 220(7), 1170–1180.
- Nanushyan, E., & Murashov, V., (2001). Plant meristem cell response to stress factors of the geomagnetic field (GMF) fluctuations. *Plant. Environ. Stress*, 204, 205.
- Naz, A., Jamil, Y., Iqbal, M., Ahmad, M. R., Ashraf, M. I., & Ahmad, R., (2012). Enhancement in the germination, growth and yield of okra (*Abelmoschus esculentus*) using pre-sowing magnetic treatment of seeds. *Indian J. Biochem. Biophys.*, 49(3), 211–214.
- Noran, R., Shani, U., & Lin, I., (1970). The effect of irrigation with magnetically treated water on the translocation of minerals in the soil. *Magn. Electr. Separ.*, 7, 109–122.
- Nossol, B., Buse, G., & Silny, J., (1993). Influence of weak static and 50 Hz magnetic fields on the redox activity of cytochrome-C oxidase. *Bio Electromagn.*, 14(4), 361–372.
- Novitskaya, G., Molokanov, D., Kocheshkova, T., & Novitskii, Y. I., (2010). Effect of weak constant magnetic field on the composition and content of lipids in radish seedlings at various temperatures. *Russ. J. Plant Physiol.*, 57(1), 52–61.

- Occhipinti, A., De Santis, A., & Maffei, M. E., (2014). Magnetoreception: An unavoidable step for plant evolution? *Trends Plant Sci.*, 19(1), 1–4.
- Paul, A. L., Ferl, R. J., & Meisel, M. W., (2006). High magnetic field induced changes of gene expression in *Arabidopsis*. *Biomagn. Res. Technol.*, 4(1), 1–10.
- Payez, A., Ghanati, F., Behmanesh, M., Abdolmaleki, P., Hajnorouzi, A., & Rajabbeigi, E., (2013). Increase of seed germination, growth and membrane integrity of wheat seedlings by exposure to static and a 10 Khz electromagnetic field. *Electromagn. Biol. Med.*, 32(4), 417–429.
- Pazur, A., & Rassadina, V., (2009). Transient effect of weak electromagnetic fields on calcium ion concentration in *Arabidopsis thaliana*. *BMC Plant Biol.*, 9(1), 1–9.
- Pittman, U., (1962). Growth reaction and magnetotropism in roots of winter wheat (Kharkov 22 MC). *Can. J. Plant Sci.*, 42(3), 430–436.
- Poinapen, D., Brown, D. C., & Beecharry, G. K., (2013). Seed orientation and magnetic field strength have more influence on tomato seed performance than relative humidity and duration of exposure to non-uniform static magnetic fields. *J. Plant Physiol.*, 170(14), 1251–1258.
- Pooam, M., Arthaut, L. D., Burdick, D., Link, J., Martino, C. F., & Ahmad, M., (2019). Magnetic sensitivity mediated by the *Arabidopsis* blue-light receptor cryptochrome occurs during flavin reoxidation in the dark. *Planta*, 249(2), 319–332.
- Portaccio, M., De Luca, P., Durante, D., Grano, V., Rossi, S., Bencivenga, U., Lepore, M., & Mita, D., (2005). Modulation of the catalytic activity of free and immobilized peroxidase by extremely low frequency electromagnetic fields: Dependence on frequency. *Bio Electromagn.*, 26(2), 145–152.
- Prajapati, R., Kataria, S., & Jain, M., (2020). Seed priming for alleviation of heavy metal toxicity in plants: An overview. *Plant Sci. Today*, 7(3), 308–313.
- Qamili, E., De Santis, A., Isac, A., Manda, M., Duka, B., & Simonyan, A., (2013). Geomagnetic jerks as chaotic fluctuations of the earth's magnetic field. *Geochem. Geophys. Geosyst.*, 14(4), 839–850.
- Radhakrishnan, R., & Kumari, B. D. R., (2012). Pulsed magnetic field: A contemporary approach offers to enhance plant growth and yield of soybean. *Plant Physiol. Biochem.*, 51, 139–144.
- Radhakrishnan, R., & Kumari, B. D. R., (2013). Influence of pulsed magnetic field on soybean (*Glycine max* L.) seed germination, seedling growth and soil microbial population. *Indian J. Biochem. Biophys.*, 50(4), 312–317.
- Radhakrishnan, R., & Kumari, B. R., (2013). Protective role of pulsed magnetic field against salt stress effects in soybean organ culture. *Plant Biosystems-An International Journal Dealing with All Aspects of Plant Biology*, 147(1), 135–140.
- Radhakrishnan, R., (2019). Magnetic field regulates plant functions, growth and enhances tolerance against environmental stresses. *Physiol. Mol. Biol. Plants*, 25(5), 1107–1119.
- Radhakrishnan, R., Leelapriya, T., & Kumari, B. D. R., (2012). Effects of pulsed magnetic field treatment of soybean seeds on Calli growth, cell damage, and biochemical changes under salt stress. *Bio Electromagn.*, 33(8), 670–681.
- Rajendra, P., Sujatha, N. H., Sashidhar, R., Subramanyam, C., Devendranath, D., Gunasekaran, B., Aradhya, R., & Bhaskaran, A., (2005). Effects of power frequency electromagnetic fields on growth of germinating *Vicia faba* L. the broad bean. *Electromagn. Biol. Med.*, 24(1), 39–54.

- Ramesh, B., Kavitha, G., Gokiladevi, S., Balachandar, R. K., Kavitha, K., Gengadharan, A. C., & Puvanakrishnan, R., (2020). Effect of extremely low power time-varying electromagnetic field on germination and other characteristics in foxtail millet (*Setaria italica*) seeds. *Bio Electromagn.*, 41(7), 526–539.
- Rathod, G. R., & Anand, A., (2016). Effect of seed magnetopriming on growth, yield and Na/K ratio in wheat (*Triticum aestivum* L.) under salt stress. *Indian J. Plant Physiol.*, 21(1), 15–22.
- Razmjoo, J., & Alinian, S., (2017). Influence of magnetopriming on germination, growth, physiology, oil and essential contents of cumin (*Cuminum cyminum* L.). *Electromagn. Biol. Med.*, 36(4), 325–329.
- Reina, F. G., & Pascual, L. A., (2001). Influence of a stationary magnetic field on water relations in lettuce seeds. Part I: Theoretical considerations. *Bio Electromagn.*, 22(8), 589–595.
- Rochalska, M., & Orzeszko-Rywka, A., (2005). Magnetic field treatment improves seed performance. *Seed Sci. Technol.*, 33(3), 669–674.
- Ružič, R., & Jerman, I., (2002). Weak magnetic field decreases heat stress in cress seedlings. *Electromagn. Biol. Med.*, 21(1), 69–80.
- Sahebamei, H., Abdolmaleki, P., & Ghanati, F., (2007). Effects of magnetic field on the antioxidant enzyme activities of suspension-cultured tobacco cells. *Bio Electromagn.*, 28(1), 42–47.
- Samani, M. A., Pourakbar, L., & Azimi, N., (2013). magnetic field effects on seed germination and activities of some enzymes in cumin. *Life Sci. J.*, 10(1), 323–328.
- Sarraf, M., Kataria, S., Taimourya, H., Santos, L. O., Menegatti, R. D., Jain, M., Ihtisham, M., & Liu, S., (2020). Magnetic field (MF) applications in plants: An overview. *Plants*, 9(9), 1139.
- Schopfer, P., (2001). Hydroxyl radical-induced cell wall loosening in vitro and *in vivo*: Implications for the control of elongation growth. *Plant J.*, 28(6), 679–688.
- Selim, A. F. H., & El-Nady, M. F., (2011). Physio-anatomical responses of drought stressed tomato plants to magnetic field. *Acta Astronaut.*, 69(7, 8), 387–396.
- Sen, A., & Alikamanoglu, S., (2014). Effects of static magnetic field pretreatment with and without PEG 6000 or nacl exposure on wheat biochemical parameters. *Russ. J. Plant Physiol.*, 61(5), 646–655.
- Shabrangi, A., & Majd, A., (2009). Effect of magnetic fields on growth and antioxidant systems in agricultural plants. *PIERS Proceedings*, 23–27.
- Shaddad, M., (1990). The effect of proline application on the physiology of *Raphanus sativus* plants grown under salinity stress. *Biol. Planta*, 32(2), 104–112.
- Shahin, M., Mashhour, A., & Abd-Elhady, E., (2016). Effect of magnetized irrigation water and seeds on some water properties, growth parameter and yield productivity of cucumber plants. *Curr. Sci. Int.*, 5(2), 152–164.
- Shine, M., Guruprasad, K., & Anand, A., (2011). Enhancement of germination, growth, and photosynthesis in soybean by pre-treatment of seeds with magnetic field. *Bio Electromagn.*, 32(6), 474–484.
- Smirnov, N., & Arnaud, D., (2019). Hydrogen peroxide metabolism and functions in plants. *New Phytol.*, 221(3), 1197–1214.
- Song, W. Y., Zhang, Z. B., Shao, H. B., Guo, X. L., Cao, H. X., Zhao, H. B., Fu, Z. Y., & Hu, X. J., (2008). Relationship between calcium decoding elements and plant abiotic stress resistance. *Int. J. Biol. Sci.*, 4(2), 116.

- Sunita, K., Lokesh, B., & Guruprasad, K., (2015). Acceleration of germination and early growth characteristics of soybean and maize after pre-treatment of seeds with static magnetic field. *J. Trop. Agric.*, 33(2 (Part II), 985–992.
- Thomas, S., Anand, A., Chinnusamy, V., Dahuja, A., & Basu, S., (2013). Magnetopriming circumvents the effect of salinity stress on germination in chickpea seeds. *Acta Physiol. Plant.*, 35(12), 3401–3411.
- Trebbi, G., Borghini, F., Lazzarato, L., Torrigiani, P., Calzoni, G. L., & Betti, L., (2007). Extremely low frequency weak magnetic fields enhance resistance of NN tobacco plants to tobacco mosaic virus and elicit stress related biochemical activities. *Bio Electromagn.*, 28(3), 214–223.
- Ul Haq, Z., Iqbal, M., Jamil, Y., Anwar, H., Younis, A., Arif, M., Fareed, M. Z., & Hussain, F., (2016). Magnetically treated water irrigation effect on turnip seed germination, seedling growth and enzymatic activities. *Inf. Process. Agric.*, 3(2), 99–106.
- Vashisth, A., & Joshi, D. K., (2017). Growth characteristics of maize seeds exposed to magnetic field. *Bio Electromagn.*, 38(2), 151–157.
- Vashisth, A., & Nagarajan, S., (2008). Exposure of seeds to static magnetic field enhances germination and early growth characteristics in chickpea (*Cicer arietinum* L.). *Bio Electromagn.*, 29(7), 571–578.
- Vashisth, A., Meena, N., & Krishnan, P., (2021). Magnetic field affects growth and yield of sunflower under different moisture stress conditions. *Bio Electromagn.*
- Vian, A., Davies, E., Gendraud, M., & Bonnet, P., (2016). Plant responses to high frequency electromagnetic fields. *BioMed Res. Int.*, 2016.
- Yano, A., Ohashi, Y., Hirasaki, T., & Fujiwara, K., (2004). Effects of a 60 Hz magnetic field on photosynthetic CO<sub>2</sub> uptake and early growth of radish seedlings. *Bio Electromagn.*, 25(8), 572–581.
- Zalama, M., & Fathalla, F., (2020). Enhancement onion seed germination and seedling vigor traits through magneto-priming techniques. *J. Plant Prod.*, 11(12), 1529–1537.
- Zhadin, M. N., (2001). Review of Russian literature on biological action of dc and low-frequency AC magnetic fields. *Bio Electromagn.*, 22(1), 27–45.

## CHAPTER 6

---

# Plant Breeding Approaches in Developing Stress Tolerance

SAMI UL-ALLAH,<sup>1\*</sup> ASAD AZEEM,<sup>1</sup> AHMAD SHER,<sup>1</sup> MUHAMMAD IJAZ,<sup>1</sup> ABDUL SATTAR,<sup>1</sup> MUHAMMAD NAEEM,<sup>2</sup> and IMRAN ULLAH<sup>3</sup>

<sup>1</sup>*College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Pakistan*

<sup>2</sup>*Department of Plant Breeding and Genetics, UCA&ES, The Islamia University of Bahawalpur, Pakistan*

<sup>3</sup>*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan*

*\*Corresponding author. E-mail: samipbg@bzu.edu.pk*

---

## ABSTRACT

Biotic and abiotic stresses are major hurdles to maximize crop production, and in climate change scenarios, the threat of these stresses is even high. Major abiotic stresses include salinity, drought, temperature, and heavy metal and major biotic stresses include plant diseases and insect-pest attacks. These stresses have resulted in severe crop losses on a global scale. Scientists have developed various techniques to alleviate the effect of these stresses for optimum crop production, like agronomic management, soil amendments, use of microorganisms, use of pesticides, integrated pest and disease management, and development of tolerant genotypes. From all the above-mentioned techniques, the development of tolerant genotypes is the most sustainable technique that does not require any input except the seed of tolerant genotype. There are two major types of breeding techniques



used to introgress stress tolerance in cultivated genotype, i.e., conventional plant breeding and molecular plant breeding, further divided into various sub-disciplines. This chapter will discuss various plant breeding approaches for the introgression of tolerance against biotic and abiotic stresses, pros and cons, limitations, and prospects.

## **6.1 INTRODUCTION**

Biotic and abiotic stresses are the leading factors of yield reduction in the major crops (Canter, 2018; Zörb et al., 2019). The major abiotic stresses are temperature, drought, and excess salt, affecting crop physiology and yield (Waqas et al., 2017; Vaughan et al., 2018; Zafar et al., 2018). The abiotic stress affects 90% of the land, which minimizes the yield up to 70% (Mantri et al., 2012). The prediction made on the crop yield models in relation to the changing climate expresses the loss in yield of many crops, including maize, rice, and wheat, which may lead to devastating and the most serious outcomes for food security (Tigchelaar et al., 2018). Through the genetic mechanism, plants become able to stand biotic and abiotic stress (Roy et al., 2014; Pradhan et al., 2019). Plant breeding approaches regarding stress tolerance in the plant make it tolerant to tackle stress with little effort (Bertoldo et al., 2014). Plant with the improved genomic structure can tackle multiple stress with less yield reduction in a variety of environments (Yumurtaci, 2015).

Transgenic approaches are the best tool to improve plant architecture against the biotic and abiotic stresses, quantity, and quality of economic products in plants (Wang et al., 2016a, b). Recombinant DNA technology is the main player to improve the yield potential, nutritional value as well as shelf life in a severe and delicate situation (Roychowdhury et al., 2013, 2014; Hasanuzzaman et al., 2013; Roychowdhury & Tah, 2013; Roychowdhury, 2014; Anumalla et al., 2015; Wani et al., 2016). Recombinant DNA technology is responsible for many beneficial roles regarding traits improvement and new varieties development with novel characteristics (Marco et al., 2015). Genetically modified (GM) plants have proline, glycine betaine, and different proteins such as molecular chaperones linked to a defense mechanism against biotic and abiotic stresses as reported in many field crops (Grobkinsky et al., 2016; Hasanuzzaman et al., 2015; Anumalla et al., 2016). Recently, genetic engineering is mostly used weapon to combat biotic and abiotic stresses in horticultural crops (Bakhsh & Hussain, 2015). In keeping

in view, the current and coming challenges in the future, a comprehensive review on biotic and abiotic stress is composed by using breeding and genetic approaches.

## **6.2 GENETIC MECHANISM OF STRESS TOLERANCE**

### **6.2.1 SALINITY**

Excess of salt leads to ionic as well as osmotic stress in plants. Salt overly sensitive pathway is a crucial mechanism in salt stress which is responsible for regulating ion homeostasis. The salt overly sensitive pathway consists of three main genes SOS2, SOS3, and SOS1 (Ji et al., 2013). Regulation of the ion homeostasis is a crucial way to tackle salt stress in which sodium ion extrusion is a basic phenomenon to make the plant tolerant to salt stress. As the level of sodium ion increases from a certain threshold level, all metabolic pathways and enzymatic mechanisms are disrupted, leading to toxicity. Calcium ions are responsible for stimulating salt overly sensitive pathway in the cytoplasm of root cell. SOS3 activates the basic calcium ion sensor and the attachment of the SOS3, and calcium ion is stimulation to activate the SOS2. But SCABP8 is an activator of SOS2 in shoots. As SOS2 is activated, it resides in the cell membrane where it activates downstream SOS1, an antiporter of the sodium ion/hydrogen ion, and its activation results in sodium-ion extrusion. Various SOS1 mutants are very effective and sensitive to salinity (Ji et al., 2013). Highly reactive potassium transporter genes are also responsible for exclusion ions. With the help of sodium ion exclusion, HKT2 works efficiently to enhance salt tolerance in the plant (Roy et al., 2014). In rice, the salt tolerance can be induced through sodium ion/hydrogen ion antiporters such as OsNHX2, OsNHX4, OsNHX1, and OsNHX3 by the sodium ion sequestration. Sodium ion/hydrogen ion antiporters activities are very high when salt is high. When the expression of OsNHX1 is high, it enhances tolerance owing to sodium ion accumulation in the vacuole of plant cells and makes transgenic rice plant tolerant. In rice, the most effective and efficient adaptation is gathering the sodium ion in old leaves of rice to tackle salt stress (Reddy et al., 2017).

A high amount of salt promotes abscisic acid (ABA) synthesis, which is responsible for closing stomata in leaves and makes plants tackle serious osmotic stress during salinity. The expression of genes in rice plant such as OsDREB1F, OsDREB2A, and OsDREB1A makes it tolerant to salt stress.

In plants PDH45, and the OsCPK12 gene maintains ROS make the plant tolerant to salt stress (Reddy et al., 2017).

### 6.2.2 DROUGHT

Osmotic stress in plants is also due to the low water availability. In drought conditions, the late embryogenesis abundant (LEA) proteins are responsible for protecting plant against desiccation. Expressions of LEA class genes results to enhance tolerance against water stress. In transgenic Arabidopsis, the gene that codes galactinol synthase involved in synthesizing oligosaccharides belongs to family raffinose to enhance tolerance against drought. During drought conditions, raffinose acts as an osmoprotectant and decreases cellular damages in plant (Shinozaki & Yamaguchi-Shinozaki, 2007). MsrB2 gene produce tolerance against drought by minimizing oxidative injury of ROS. The rice drought-tolerant gene CaMsrB2 is taken from *Capsicum annuum*, making transgenic rice drought tolerant (Nahar et al., 2016).

### 6.2.3 HIGH TEMPERATURE

Cell membrane thermostability is a checkpoint of heat stress tolerance in plants. In many types of grass, the genetic cause of many heat shock proteins (HSPs) has been identified by co-segregation and QTL analyzes. In grasses, tolerance to heat stress is not regulated by a single gene (Maestri et al., 2002). Heat stress in plants leads to express genes related to HSPs. Therefore, heat shock factors get attached to Heat Shock Elements' binding site in the promoters of heat shock genes, including the HSPs' expression on high-temperature stress. HSPs play an important role as chaperones and are involved in saving the plant's intracellular proteins by keeping intact the functioning and structural stability of plant cells. Based on molecular weight, HSPs are classified into various groups, viz., HSP20, HSP70, HSP100, HSP60, and HSP90. HSP with low molecular weight showed diversity among other HSPs (Hasanuzzaman et al., 2013).

In Arabidopsis HSP101, and HSP70 proteins are responsible for generating tolerance in plant against heat stress. In the wheat plant following genes, HSP101 members, Tahsp101c, Tahsp101b, and Tahsp101a, have been introduced to develop tolerance against high-temperature stress (Maestri et al., 2002). H<sub>2</sub>O<sub>2</sub>-responsive ANP1/NPK1 gene in tobacco can develop heat stress in plants. APX1 gene in barley is responsible for heat tolerance compared to

barley without this gene (Hasanuzzaman et al., 2013). Arabidopsis HSFA1a and HSFA1b genes are tolerant to heat stress. In tomato HSFA1 gene is able to tolerate high temperature stress (Hasanuzzaman et al., 2013).

#### **6.2.4 LOW TEMPERATURE**

A lot of genes in the plant are responsible for inducing cold stress tolerance in plants. Fatty acids desaturation is essential for the performance of membrane in low-temperature stress. LOS4 and AtNUP160 genes of Arabidopsis that send RNA to cytoplasm from nucleus are very important to tackle low-temperature stress in plants (Sanghera et al., 2011).

### **6.3 GENETIC MECHANISM AGAINST BIOTIC STRESSES**

Various genes in plants are tolerant to biotic stress, especially by pathogens (Mamgain et al., 2013). ABA-independent dehydration-responsive DREB2A is directly involved in developing disease resistance in plants (Agarwal et al., 2006). OsDREB1B gene in Transgenic tobacco plants is responsible for tackling plants' disease (Gutha & Reddy, 2008). OsEREBP1 gene is responsible for the fungus *Magnaporthe grisea*. However, transcriptome analysis showed that it belongs to WRKY and NAC groups (Jisha et al., 2015). The SIAREB1 gene has increased the expression of PR proteins in plant (Orellana et al., 2010). ATAF1 gene increases tolerance against *Blumeria graminis* f. sp. *Graminis* but decreased tolerance against *Botrytis cinerea*, *Pseudomonas syringae* and *Alternaria brassicicola* (Puranik et al., 2012). OsNAC6 gene in rice plant is responsible for developing resistance against rice blast (Nakashima et al., 2007).

#### **6.3.1 INSECT PEST RESISTANCE**

In the okra plant, the target gene Cry1AC causes resistance against fruit borer and stem borer (Narendran et al., 2013). A sbtCry1AC gene in Planch is responsible for developing *Oraesia excavate* resistance (Zhang et al., 2015a, b, c). In Daisy, resistance against aphids is induced via CmWRKY48 gene introduction, which plays many functions in favor of plants to control aphids (Li et al., 2015).

### 6.3.2 DISEASE RESISTANCE

In strawberry, resistance against crown gall disease is developed by introducing the  $\beta$ -1,3-glucanase gene, which made plant resistant to fungal disease (Mercado et al., 2015). The resistance against conidial growth in guava plants is developed by the Endochitinase gene taken from *Trichoderma* (Mishra et al., 2016).

In tobacco, Tomato yellow leaf curl China virus resistance is induced by Zinc finger nuclease (Chen et al., 2014) and resistance against Tobacco curly shoot virus is introduced by Transcription activator-like effector nuclease (Cheng et al., 2015). In tobacco, plant tolerance against Tomato leaf curl Yunnan virus is developed via Transcription activator-like effector nuclease (Ali et al., 2015). In *Arabidopsis thaliana* and *Nicotiana benthamiana* the gene virus dsDNA (A7, B7, and C3 regions) resistant to beet severe curly top virus (Ji et al., 2015) and gene eIF(iso)4E is responsible for resistance against Turnip mosaic virus in *Arabidopsis thaliana* (Pyott et al., 2016). In rice resistance against Blast is developed by gene OsERF922 (Wang et al., 2016a, b). In wheat resistance against Powdery mildew is developed by TaMLO-A1, TaMLO-B1, and TaMLOD1 (Wang et al., 2014). In the cucumber plant, eIF4E is responsible for creating resistance against Zucchini yellow mosaic virus, Cucumber vein yellowing virus, and Papaya ringspot mosaic virus type-W (Chandrasekaran et al., 2016). In cotton plants, CLCuD IR and Rep regions are resistant to cotton leaf curl disease (Iqbal et al., 2016).

## 6.4 CONVENTIONAL PLANT BREEDING APPROACHES

Plant breeding approaches play an essential role in developing biotic and abiotic stress tolerance in plants, making them tackle various stress in plants' lives at different growth and developmental stages. Many methods are applicable to develop resistance in plants against many diseases, low, and high temperature, and salt in plants. For instance, mungbean resistance against biotic stresses has been developed by breeding approaches like introduction, hybridization, and selection (Pratap et al., 2012).

### 6.4.1 INTRASPECIFIC PLANT BREEDING APPROACHES

Many studies showed that plants' resistance against various stresses like biotic and abiotic stresses could be developed by intraspecific hybridization

in plants. In mungbean, many varieties against biotic (mostly powdery mildew and mungbean yellow mosaic disease) and abiotic stresses have been developed through intraspecific approaches in plant breeding (Pratap et al., 2014a, 2015; Singh et al., 2017). Hybridization is a powerful tool in varietal development against biotic and abiotic stresses. The development of stress-tolerant varieties leads to boost agricultural production in less and reasonable amounts. For example, a cross between PS 16 X BM4 leads to develop mungbean variety PKV AKM which is highly resistant to stresses. Through IPM 99–125 and Pusa Bold 2, the most resistant variety of mungbean against diseases, especially mungbean yellow mosaic disease, has been developed, showing about 82.52% of preharvest spouting value (Lamichane et al., 2017). KM 2328 is developed by the cross between KM 2241 and HUM 16 in 2018, which is resistant to MYMD, WB, and anthracnose (Coordinated Research Project on MULLaRP, Project Coordinators Report, 2018). IPM 205-7 is highly tolerant to high temperatures and matures earlier than others (Pratap et al., 2013).

#### **6.4.2 INTERSPECIFIC PLANT BREEDING APPROACHES**

Inter-specific plant breeding approaches are of great importance to develop resistance against biotic and abiotic stresses in plants. The exotic and wild cultivars of mungbean can create resistance against various stresses and improve yield quality and quantity (Pratap et al., 2015). Inter-specific crosses are the complex and complicated process of gene transmission to create resistant varieties in the plant, such as mungbean (Pratap et al., 2018). Improved F7 RILS and subsequent advanced generation of mungbean is obtained by intercrossing between V2802 and NM94, which is resistant against bruchid damage in Taiwan (Schafleitner et al., 2016). Intercrossing between urdbean and mungbean results in variety resistant to MYMD (Gill et al., 1983; Lekhi, 2017).

### **6.5 MOLECULAR PLANT BREEDING APPROACHES**

#### **6.5.1 QTL MAPPING**

QTL mapping is another molecular approach used when more than one gene is responsible for a trait in a specific chromosome location. The basic principle of QTL mapping is the segregation of genes during meiosis. Using

the recombination frequency, the gap between different markers can be calculated; therefore, the position of different QTLs in a specific genome can also be calculated (Collard et al., 2005). In the wheat plant about 10 QTLs for yellow rust resistance have been identified by the help of QTLs mapping. Among these 10 QTLs, the 05 major QTLs are QYr.sgi-4A.1, Lr34/Yr18, QYr.sgi-2B.1a, QYr.sgi-4A.2, and QYr.sgi-2B.1 (Chu et al., 2009).

### **6.5.2 MARKER-ASSISTED SELECTION**

In plants, breeding approaches DNA markers to play an essential role in identifying and selecting plants with the suitable trait (Roychowdhury, 2014; Roychowdhury et al., 2014). Owing to a large number, these DNA markers are used in a wide range to study and identify plants and these markers are neutral as they are present in the coding region. The DNA markers which are used in a wide range are simple sequence repeats (SSR), restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), and random amplified polymorphic DNA (RAPD) (Collard et al., 2005). The marker-assisted selection also plays an important role in the germplasm screening for resistant genes (Simon-Mateo & Garcia, 2011). In sorghum plants, genes responsible for smut resistance were identified by RAPD and RFLP markers (Oh et al., 1994). Marker-assisted recurrent selection increases the frequency of genes of interest (GOI) when many genes are involved in a specific trait expression. By using Marker-assisted recurrent selection in drought conditions, genetic gain for higher grain yield up to 51 kg ha<sup>-1</sup> year<sup>-1</sup> of maize was produced in the region of sub-Saharan Africa (Beyene et al., 2016).

### **6.5.3 DEVELOPMENT OF TRANSGENICS**

#### **6.5.3.1 TRANSGENIC APPROACHES FOR BIOTIC STRESS**

Biotic stress like bacteria, viruses, insect pests, etc. Many techniques have been practiced, but not too many satisfactory results are shown, but these stresses are possible due to the transgenic approaches. In subsections, we have put in a nutshell the recent success story of the crop with biotic stress resistance and tolerance with the help of novel transgenic techniques.

### **6.5.3.1.1 Transgenic Approaches for Insect Pest Resistance**

The insects and pests are the most diverse form of biotic stress in yield destruction and increment in their number throughout their life cycle. They are the major factor of the yield reduction of the food crops and are leading to cause severe issues in the provision of food to the increasing world population day by day (Douglas, 2018). The major two groups of the damages in crops of insect pests are direct and indirect damages. Direct damages caused by insect pests are plant cell and tissues death owing to disgustingly dirty filled, accidental death of floral tissues and organ and the whole process lead to the flourishing of the plant pathogen reproduction as well as distribution. But the indirect damages are the fruits' bruising which causes an increase in expenses of management and reduction in yield (Pappas et al., 2017). Recently reported that the yield loss of about 20% of horticultural crops globally. Research show that about 0.5% of insect pest is responsible for major issue to human beings so that they are called pest and belong to the Lepidoptera class (Zaidi et al., 2017). Therefore, transgenic approaches are the best way to tackle the pest of class Lepidoptera.

Transgenic approaches are the most appropriate and effective method to tackle the insect pest with a high degree of success and in hand sum cost (Majeed et al., 2018). In 1987 the 1<sup>st</sup> transgenic insect-resistant tomato plant was developed by utilizing the Cry gene from bacterium *Bacillus thuringiensis*, which showed tolerance against *Heliothis virescens* and *Spodoptera litura*. Mostly vegetables crops such as Bringle and chili are affected by the class Lepidoptera (de Castro et al., 2017). *Bt* eggplant showing cry1Ab and cry1Ac has produced and shows tolerance to the members of class Lepidoptera (Biswas et al., 2018). Transgenic cabbage line DTC 507 transformed having cry1Ab, and cry1B expressed tolerance to *Plutella xylostella* (Parmar et al., 2017). The okra cry1Ac gene is introduced, showing tolerance against the *Earias vittella* (fruit borer) (Jan et al., 2015). Transgenic strawberries show tolerance against vine weevil and aphids due to the cowpea trypsin inhibitor (*CpTi*) gene (Singh et al., 2018). Transgenic crop plants showing proteinase inhibitor genes are the most effective to tackle root-knot nematode (*Meloidogyne incognita*) by checking their metabolism (Zhang et al., 2015a, b, c). Effective resistance has been developed against root-knot nematode in many crop plants like tomato, eggplant, and potato (Seow-Neng et al., 2017).



#### **6.5.3.1.2 Transgenic Approaches for Disease Resistance**

The next yield-limiting factor is a disease that causes the major loss of production of crops. The stress causes the destruction of crop plant from germination to maturity and plant is always in the state of dilemma to combat the disease-causing organisms. The best way to introduce disease resistance in the plant is through Transgenic approaches. The gene-like pathogenesis-related (PR) genes, glucanase, and chitinase can effectively disintegrate the cell wall of disease-causing organisms against fungal and bacterial and viral diseases (Bill et al., 2016).

Similarly, the disease resistance can be introduced with the help of systemic acquired resistance (SAR)-related genes responsible for expressing many downstream genes like pathogenesis-related genes (Chen et al., 2017). Transgenic orange has been developed, which showed tolerance to *Phytophthora citrophthora* by introducing the tomato PR-5 gene (Takemoto & Mizuno, 2016). Transgenic tomato shows tolerance to Tomato mosaic virus (TMV), which has been developed by introduction of non-expression of pathogenesis-related (NPR-1) gene taken from *Arabidopsis thaliana* (Bubici et al., 2017).

Transgenic tomato showed tolerance against bacterial wilt and fusarium wilt owing to the NPR1 gene expression. Transgenic apple overexpressing NPR1 gene is resistant to apple cedar rust due to the attack of *Gymnosporangium juniper-Virginiana* and *V. inaequalis*, but blight disease of apple is owing to *Erwinia amylovora* (Thakur et al., 2018). Transgenic plant having Chitinase gene is tolerant against many fungal diseases such as tobacco ChiC gene in carrot (Su et al., 2015). Similarly, transgenic lines of potato have been developed by introducing the chitinase gene from *P. vulgaris* to tackle early blight in potatoes (Chen et al., 2018).

Transgenic guava has been developed by introducing the endochitinase gene from *Trichoderma*, which is responsible for the resistance against *Fusarium oxysporum* (Kumar et al., 2016). Recently, CRISPR Cas9 and RNAi have been widely used to overcome the issues regarding biotic and abiotic stress (Rani et al., 2016).

#### **6.5.3.1.3 Transgenic Approaches for Virus Resistance**

Viruses are the biotic agent that badly affects crop yield and physiology, and traditional breeding methods cannot tackle viral diseases (Trebicki et al.,

2015). Transgenic approaches improve crop genetic makeup by introducing resistant genes against stresses (Mishra et al., 2016). Transgenic strawberry is resistant to Strawberry mild yellow edge potexvirus (Silva et al., 2015). Similarly, the coat protein gene in transgenic chili has made chili resistant to many diseases caused by viruses (Praveen et al., 2017). The virus coat protein gene in *Citrullus lanatus* has made Zucchini yellow mosaic virus (Harth et al., 2017). p transgenic tomato developed by RNAi technique has made tomato resistant to tomato leaf curl virus.

### 6.5.3.2 TRANSGENIC APPROACHES FOR ABIOTIC STRESS

#### 6.5.3.2.1 Transgenic Approaches for Drought Stress

In plants, many regulatory genes, including transcription factors (TFs), can express a lot of downstream and many other genes for abiotic stress tolerance (Marco et al., 2015). For transgenic approaches, many transcription factors such as MYB, NAC, and MYC have been used for abiotic stress tolerance in crop plants (Marco et al., 2015). Transgenic tomato having gene from *Arabidopsis* C-repeat binding factor/dehydration-responsive element-binding in the expression of 35s, and nos showed tolerance to drought stress in comparison to the non-transgenic tomato (Kidokoro et al., 2015). In the same way, transgenic apple plants have shown tolerance against drought stress due to the expression of the MYB TFs compared to the non-transgenic apple (Buti et al., 2018). Similarly, transgenic grapevines with the DREB1b gene have shown tolerance against drought stresses than the non-transgenic grapevine.

WRKY TFs are the other set of the TFs which showed the positive regulated abiotic stress in crop plants, and these are exploited in a wide range against abiotic stress tolerance (Jiang et al., 2017). Many types of research showed that WRKY TFs are involved in creating drought stresses tolerant plants. For instance, transgenic *Chrysanthemum morifolium* plants are drought tolerant due to the ABA-mediated pathway, which activates many downstream genes like DREB1A, MYB2, and ABI2. In other research, transgenic bananas having the WRKY71 gene had made tolerant to much abiotic stress (Zhou et al., 2015). Similarly, NAC TFs having three TFs (no apical meristem (N)/*Arabidopsis* transcription activator factor (A)/cup-shaped cotyledon (C)) are used to develop transgenic crop plants which are tolerant to a lot of abiotic stress (Hong et al., 2016). In many studies, the development of the transgenic

rice having introduced genes, i.e., OsNAC6 SNAC1, and SNAC2, is tolerant to rice plants' drought stress compared to the non-transgenic rice (Todaka et al., 2015). Research showed that transgenic plants with the TaNAC67 gene are tolerant to drought stress and update physiology and metabolism (Sharma et al., 2017).

A set of hydrophilic proteins like LEA proteins are responsible for the multiple abiotic stress like salt, heat, drought, and cold stresses in crop plants (Banerjee & Roychoudhury, 2016). Therefore, transcription factors are the most vital element to induce abiotic stress in a crop plant by transgenic approaches.

#### **6.5.3.2.2 Transgenic Approaches for Heat Stress**

Heat stress is vital abiotic stress which affects the crop yield and physiological traits of crop plants (Zhu, 2016). Heat stress decreases the rate of photosynthesis, damages the cell membranes, and decreases the activity of reactive oxygen species (Dwivedi et al., 2016). The level of reactive oxygen species exceeding a certain limit will cause protein denaturation and decrease protein homeostasis. Transgenic approaches have made plant efficient and effective reactive oxygen species scavenging ability as it is a part of the signaling pathways as their level exceed the limit. These are effectively scavenged via antioxidant enzymes like glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX) (Fancy et al., 2017). ROS-scavenging enzymes in transgenic plants is a way to tackle high-temperature stress which causes oxidative disintegration in crop plants. Transgenic apple plants having cytosolic ascorbate peroxidase (cAPX) can minimize cell membrane damage and increase the photosynthesis rate (Zandalinas et al., 2018). Transgenic tomato plants comprising the cAPX gene can tolerate high-temperature stress up to 40°C in field conditions with minimum heat stress oxidative injury compared to the non-transgenic tomato plant (Sadiq & Akram, 2018). In many types of research, transgenic tomatoes having polyamine expressed tolerance against the heat stress; expression of the S-adenosyl-I-methionine decarboxylase from *Saccharomyces cerevisiae* led to the manufacturing of polyamines which made tomato plant tolerant to high-temperature stress (Parmar et al., 2017). Similarly, the Transgenic potato plant having Cu/Zn SOD taken from the okra plant can tackle high-temperature stress by activating many reactive oxygen species – scavenging enzymes compared to the non-transgenic potato (Sadiq & Akram, 2018).

Transgenic plants with HSPs are tolerant to heat stress by minimizing the protein denaturation with the help of protein homeostasis (Jacob et al., 2017). Many studies showed that the expression of the HSPs in Arabidopsis plant and tomato plant has made it resistant to high-temperature stress. In Glycine max, the expression of the heat shock factor made it tolerant through the stimulation of the many downstream genes' expression involved in heat defense system (Zhang et al., 2015a, b, c). Likewise, the introduction of VpHSF1 gene in tobacco plants increased the capacity to tolerate high-temperature stress as compared to nontransgenic tobacco plants (Hu et al., 2016). The points mentioned above showed that the heat shock factor genes are the best player to confer heat stress. Therefore, abiotic stress tolerance can be induced efficiently by transgenic approaches.

#### 6.5.3.2.3 TRANSGENIC APPROACHES FOR SALT STRESS TOLERANCE

Salt accumulation in agricultural land is serious issue crop plants face regarding disturbed physiology and retarded growth. Salt accumulation is very severe abiotic stress affecting crop yield globally (Bless et al., 2018). Recently reported that approx. 77 million hectare of agricultural land is not suitable for cultivation due to excessive salt accumulation (Bless et al., 2018).

Generally, the soil with more than 4 dS m<sup>-1</sup> electrical conductivity (EC) and 40 mM sodium chloride with 15% exchangeable sodium rate is called saline soil (Singh, 2015). The main agents involved in soil salinization are low precipitation, saline water irrigation, poor agronomic practices, and weathering of rocks. If not checked, more than 50% of agricultural land will be saline land up to 2050 (Singh, 2018). As other abiotic stresses have a very complicated regulatory setup, saline stress is complex in regulation and is controlled by many genes (Munns & Gilliam, 2015). Some studies showed that plants tolerant to abiotic stress could also manufacture many pathogenic proteins to mitigate the effect of stress (Negrao et al., 2017). Many pathogenesis-related proteins among these Osmotin proteins can generate innate immunity in plants against abiotic stresses (Wan et al., 2017). Osmotic genes taken from tobacco plants are introduced in strawberry plants showed tolerance against salt stress (Sripriya et al., 2017). Transgenic chili with the Osmotin genes is tolerant to salt stress up to 300 mM NaCl, leading to increased antioxidant enzymes activities and photosynthesis rate (Ullah et al., 2018).

Transgenic cherry tomato having D-galacturonic acid reductase gene could bear salt stress up to 200 mM and improve the stress defense

mechanism's performance in plants (Lim et al., 2016). The expression of sodium ion/hydrogen ion antiporter genes in transgenic chili and tomato plants has improved salt defense pathways via accumulation of proline and glycine betaine and increased water amount to tackle salinity in the plant (Buller et al., 2016) (Tables 6.1 and 6.2).

**TABLE 6.1** Different Gene Function Against Different Biotic and Abiotic Stresses

Gene	Organism	Function	References
OsERF922	<i>Oryza sativa</i>	Rice blast resistance	Wang et al. (2014)
PIMP1	<i>Triticum aestivum</i>	Drought stress resistance	Liu et al. (2011)
NAC4	<i>Triticum aestivum</i>	Salt stress resistance and biotic stress	Xia et al. (2010)
BIERF1-4	<i>Oryza sativa</i>	Cold, drought stress tolerance	Cao et al. (2006)
AREB1	<i>Lycopersicon esculantum</i>	Resistance against salt and water scarcity	Orellana et al. (2010)
Cystatin gene (OC-IDD86)	Potato	<i>M. incognita</i> resistance	Lilley et al. (2004)
MAPK1	<i>B. napus</i>	Drought tolerance	Weng et al. (2014)
TTG2	<i>B. napus</i>	Salt tolerance	Li et al. (2015)
ACC-deaminase	<i>B. napus</i>	Improve excessive salt bearing capacity	Sergeeva et al. (2006)
YHem1	Yeast	Improve excessive salt bearing capacity	Sun et al. (2015)
TaMLO-A1	<i>Triticum aestivum</i>	Powdery mildew resistance	Wang et al. (2014)
OsSWEET11	<i>Oryza sativa</i>	Bacterial blight resistance	Jiang et al. (2013)
OsALS	<i>Oryza sativa</i>	Herbicide-resistant	Li et al. (2016)
Os SAPK2	<i>Oryza sativa</i>	Drought tolerance	Lou et al. (2017)

**TABLE 6.2** Transgene Against Biotic and Abiotic Stresses

Gene	Source Organism	Function	Reference
AREB1	<i>Lycopersicon esculantum</i>	Tolerant to salt and low water stress	Orellana et al. (2010)
ERF3	Glycine max	Tolerant to salt and low water stress	Zhang et al. (2009)
MYB4	<i>Oryza sativa</i>	Tolerant to low temperature stress	Vannini et al. (2004, 2007)
PIMP1	<i>Triticum aestivum</i>	Tolerant to salt and low water stress	Liu et al. (2011)

## 6.6 CONCLUSION AND FUTURE PROSPECTS

Biotic and abiotic stresses in crop plants are big challenges from a decade to overcome to feed humanity. Different conventional and molecular techniques were used to improve crop yield and enhance overall crop productivity to overcome this problem. The problem with breeding crops for biotic stresses is that insect and disease species, and even races, differ from region to region, making it impossible for a single cultivar to retain long-term resistance. Pathogen recombination is another issue linked with breeding for biotic stressors, as evidenced by the emergence of the Burewala CLCV strain in Pakistan and the Ug99 rust pathogen in Africa. In this context, one cultivar cannot control both pathogens, so the cultivar developed from breeding varies from area to area. While discussing about abiotic stresses, different biotic stresses affect plants in different ways. Some abiotic stresses are linked with each other. Likewise, heat stress and drought stress are linked. When plants suspect heat stress, there are maximum chances of drought stress to the plant. To overcome these biotic and abiotic stresses, many plant breeders should be encouraged to reintroduce methods like DH, broad hybridization, and mutagenesis into their breeding programs. On the other hand, molecular techniques like QTL mapping and marker-assisted breeding can develop cultivars. It's critical for the breeders with as many biotechnology tools as possible because each stress-crop situation is unique, requiring one or a combination of specialized biotechnological approaches to effectively address them.

In general, obtaining more thorough basic information of plant-stress interactions will aid in a better understanding of how to alleviate abiotic and biotic stressors and maybe allow for improved forecasts of plant response. We predicted that in the near future, there would be a growing interest in studying the processes of plant stress tolerance. Overall, we should need well-elaborated, week-designed, and long-term field-related studies are needed to assess the abiotic and biotic stressors.

## KEYWORDS

- **amplified fragment length polymorphisms**
- **cowpea trypsin inhibitor**
- **pathogenesis**
- **random amplified polymorphic**
- **restriction fragment length polymorphisms**
- **simple sequence repeats**

## REFERENCES

- Ali, Z., Abulfaraj, A., Idris, A., Ali, S., Tashkandi, M., & Mahfouz, M. M., (2015). CRISPR/Cas9-mediated viral interference in plants. *Genome Biol.*, 16, 238.
- Banerjee, A., & Roychoudhury, A., (2016). Group II late embryogenesis abundant (LEA) proteins: Structural and functional aspects in plant abiotic stress. *Plant Growth Regul.*, 79(1), 1–7.
- Bless, A. E., Colin, F., Crabit, A., Devaux, N., Philippon, O., & Follain, S., (2018). Landscape evolution and agricultural land salinization in coastal area: A conceptual model. *Sci. Total Environ.*, 625, 647–656.
- Bulle, M., Yarra, R., & Abbagani, S., (2016). Enhanced salinity stress tolerance in transgenic chilli pepper (*Capsicum annuum* L.) plants overexpressing the wheat antiporter (TaNHX2) gene. *Mol. Breed.*, 36, 36.
- Buti, M., Pasquariello, M., Ronga, D., Milc, J. A., Pecchioni, N., Pucciariello, C., Perata, P., & Francia, E., (2018). Transcriptome profiling of short-term response to chilling stress in tolerant and sensitive *Oryza sativa* ssp. japonica seedlings. *Funct. Integr. Genomic*, 18(6), 627–644.
- Butler, N. M., Baltes, N. J., Voytas, D. F., & Douches, D. S., (2016). Geminivirus-mediated genome editing in potato (*Solanum tuberosum* L.) using sequence-specific nucleases. *Front Plant Sci.*, 7, 1045. <https://doi.org/10.3389/fpls.2016>.
- Canter, L. W., (2018). *Environmental Impact of Agricultural Production Activities*. Broken Sound Parkway NW: CRC Press. doi: 10.1201/9781351071796.
- Cao, Y. F., Song, F. M., Goodman, R. M., & Zheng, Z., (2006). Molecular characterization of four rice genes encoding ethylene-responsive transcriptional factors and their expressions in response to biotic and abiotic stress. *Journal of Plant Physiology*, 163, 1167–1178.
- Chen, J., Piao, Y., Liu, Y., Li, X., & Piao, Z., (2018). Genome-wide identification and expression analysis of chitinase gene family in *Brassica rapa* reveals its role in clubroot resistance. *Plant Sci.*, 270, 257–267.
- Chen, S., Li, X., Lavoie, M., Jin, Y., Xu, J., Fu, Z., & Qian, H., (2017). Diclofop-methyl affects microbial rhizosphere community and induces systemic acquired resistance in rice. *J. Environ. Sci.*, 51, 352–360.
- Cheng, X., Li, F., Cai, J., Chen, W., Zhao, N., Sun, Y., et al., (2015). Artificial TALE as a convenient protein platform for engineering broad-spectrum resistance to begomoviruses. *Viruses*, 7, 4772–4782.
- Duan, X. G., Song, Y. J., Yang, A. F., & Zhang, J. R., (2009). The transgene pyramiding tobacco with betaine synthesis and heterologous expression of AtNHX1 is more tolerant to salt stress than either of the tobacco lines with betaine synthesis or AtNHX1. *Physiologia Plantarum*, 135, 281–295.
- Dwivedi, S. L., Ceccarelli, S., Blair, M. W., Upadhyaya, H. D., Are, A. K., & Ortiz, R., (2016). Landrace germplasm for improving yield and abiotic stress adaptation. *Trends Plant Sci.*, 21(1), 31–42.
- Fancy, N. N., Bahlmann, A. K., & Loake, G. J., (2017). Nitric oxide function in plant abiotic stress. *Plant Cell Environ.*, 40(4), 462–472.
- Harth, J. E., Simmons, H. E., & Stephenson, A. G., (2017). Vertical infection of zucchini yellow mosaic virus via pollen transmission occurs at a lower frequency than ovule transmission. *Eur. J. Plant Pathol.*, 147(3), 717–720.

- Hu, X. L., Liu, R. X., Li, Y. H., Wang, W., Tai, F. J., Xue, R. L., & Li, C. H., (2010). Heat shock protein 70 regulates the abscisic acid-induced antioxidant response of maize to combined drought and heat stress. *Plant Growth Regulation*, 60, 225–235.
- Hu, Y., Han, Y. T., Zhang, K., Zhao, F. L., Li, Y. J., Zheng, Y., Wang, Y. J., & Wen, Y. Q., (2016). Identification and expression analysis of heat shock transcription factors in the wild Chinese grapevine (*Vitis pseudo reticulata*). *Plant Physiol. Biochem.*, 99, 1–10.
- Jacob, P., Hirt, H., & Bendahmane, A., (2017). The heat-shock protein/chaperone network and multiple stress resistance. *Plant Biotechnol. J.*, 15(4), 405–414.
- Jiang, J., Ma, S., Ye, N., Jiang, M., Cao, J., & Zhang, J., (2017). WRKY transcription factors in plant responses to stresses. *J. Integr. Plant Biol.*, 59(2), 86–101.
- Jiang, M., Jiang, J. J., He, C. M., & Guan, M., (2016). Broccoli plants over-expressing a cytosolic ascorbate peroxidase gene increase resistance to downy mildew and heat stress. *J. Plant Pathol.*, 1, 413–420.
- Jiang, W. Z., Zhou, H. B., Bi, H. H., Fromm, M., Yang, B., & Weeks, D. P., (2013). Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic Acids Res.*, 41, e188. doi: 10.1093/nar/gkt780.
- Kidokoro, S., Watanabe, K., Ohori, T., Moriwaki, T., Maruyama, K., Mizoi, J., et al., (2015). Soybean DREB 1/CBF-type transcription factors function in heat and drought as well as cold stress-responsive gene expression. *Plant J.*, 81(3), 505–518.
- Kumar, S. A., Kumari, P. H., Jawahar, G., Prashanth, S., Suravajhala, P., Katam, R., Sivan, P., et al., (2016). Beyond just being foot soldiers—osmotin like protein (OLP) and chitinase (Chi11) genes act as sentinels to confront salt, drought, and fungal stress tolerance in tomato. *Environ. Exp. Bot.*, 132, 53–65.
- Li, T., Liu, B., Spalding, M. H., Weeks, D. P., & Yang, B., (2012). High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nat. Biotechnol.*, 30, 390–392. doi: 10.1038/nbt.
- Lim, M. Y., Jeong, B. R., Jung, M., & Harn, C. H., (2016). Transgenic tomato plants expressing strawberry D-galacturonic acid reductase gene display enhanced tolerance to abiotic stresses. *Plant Biotechnol. Rep.*, 10(2), 105–116.
- Lou, D., Wang, H., Liang, G., & Yu, D., (2017). OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice. *Front. Plant Sci.*, 8, 993. doi: 10.3389/fpls.2017.00993.
- Mantri, N., Patade, V., Penna, S., Ford, R., & Pang, E., (2012). Abiotic stress responses in plants: Present and future. In: *Abiotic Stress Responses in Plants* (New York: Springer) (pp. 1–19). doi: 10.1007/978-1-4614-0634-1\_1.
- Marco, F., Bitrián, M., Carrasco, P., Rajam, M. V., Alcázar, R., & Tiburcio, A. F., (2015). Genetic engineering strategies for abiotic stress tolerance in plants. In: *Plant Biology and Biotechnology* (pp. 579–609). Springer, New Delhi.
- Mercado, J. A., Barcelo, M., Pliego, C., Rey, M., Caballero, J. L., MunozBlanco, J., Ruano-Rosa, D., et al., (2015). Expression of the b-1,3 glucanase gene bgn13,1 from *Trichoderma harzianum* in strawberry increases tolerance to crown rot diseases but interferes with plant growth. *Transgenic Res.*, 24, 979–989.
- Mishra, J., Singh, R., & Arora, N. K., (2017). Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. *Front Microbiol.*, 8, 1706.
- Mishra, M., Jalil, S. U., Mishra, R. K., Kumari, S., & Pandey, B. K., (2016). In vitro screening of guava plantlets transformed with endochitinase gene against *Fusarium oxysporum* f. sp. psidii. *Czech J. Genet Plant Breed.*, 52, 6–13.



- Munns, R., & Gilliam, M., (2015). Salinity tolerance of crops—what is the cost? *New Phytol.*, 3, 668–673.
- Narendran, M., Deole, S. G., Harkude, S., Shirale, D., Nanote, A., Bihani, P., Parimi, S., et al., (2013). Efficient genetic transformation of okra (*Abelmoschus esculentus* (L.) Moench) and generation of insect-resistant transgenic plants expressing the cry1Ac gene. *Plant Cell Rep.*, 32, 1191–1198.
- Negrão, S., Schmöckel, S. M., & Tester, M., (2017). Evaluating physiological responses of plants to salinity stress. *Ann. Bot.*, 119(1), 1–11.
- Orellana, S., Yanez, M., Espinoza, A., Verdugo, I., Gonzalez, E., Ruiz-Lara, S., & Casaretto, J. A., (2010). The transcription factor SIAREB1 confers drought, salt stress tolerance and regulates biotic and abiotic stress-related genes in tomato. *Plant, Cell and Environment*, 33, 2191–2208.
- Parmar, N., Singh, K. H., Sharma, D., Singh, L., Kumar, P., Nanjundan, J., Khan, Y. J., Chauhan, D. K., & Thakur, A. K., (2017). Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: A comprehensive review. *3Biotech*, 7(4), 239.
- Rani, R., Yadav, P., Barbadikar, K. M., Baliyan, N., Malhotra, E. V., Singh, B. K., Kumar, A., & Singh, D., (2016). CRISPR/Cas9: A promising way to exploit genetic variation in plants. *Biotechnol. Lett.*, 38(12), 1991–2006.
- Roychowdhury, R., & Tah, J., (2013). Mutagenesis – a potential approach for crop improvement. In: Hakeem, K. R., Ahmad, P., & Ozturk, M., (eds.), *Crop Improvement – New Approaches and Modern Techniques* (pp. 149–187). Springer, Boston.
- Roychowdhury, R., (2014). *Crop Improvement in the Era of Climate Change* (p. 496). IK International Publishing House, New Delhi.
- Roychowdhury, R., Karmakar, J., Karmakar, J., Adak, M. K., & Dey, N., (2013). Physio-biochemical and microsatellite-based profiling of lowland rice (*Oryza sativa* L.) landraces for osmotic stress tolerance. *Am. J. Plant Sci.*, 4(12), 52.
- Roychowdhury, R., Khan, M. H., & Choudhury, S., (2018). Arsenic in rice: An overview on stress implications, tolerance and mitigation strategies. In: Hasanuzzaman, M., Nahar, K., & Fujita, M., (eds.), *Plants Under Metal and Metalloid Stress* (pp. 401–415). Springer, Singapore.
- Roychowdhury, R., Khan, M. H., & Choudhury, S., (2019). Physiological and molecular responses for metalloid stress in rice - a comprehensive overview. In: Hasanuzzaman, M., Fujita, M., Nahar, K., & Biswas, J., (eds.), *Advances in Rice Research for Abiotic Stress Tolerance* (pp. 341–369). Woodhead Publishing/ Elsevier, London.
- Roychowdhury, R., Taoutaou, A., Hakeem, K. R., Gawwad, M. R., & Tah, J., (2014). Molecular marker- assisted technologies for crop improvement. In: Roychowdhury, R., (ed.), *Crop Improvement in the Era of Climate Change* (pp. 241–258). IK International Publishing House, New Delhi.
- Sadiq, M., & Akram, N. A., (2018). Field performance of transgenic drought-tolerant crop plants. In: *Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants* (pp. 83–102).
- Sharma, R., Sudan, R. S., Kumari, S., Salgotra, R. K., & Singh, R., (2017). Research article terminal heat stress-responsive genes analysis in heat susceptible HDR77 genotype of wheat (*Triticum aestivum* L.) by using semi-quantitative RTPCR. *Electron J. Plant Breed.*, 8(4), 1124–1132.

- Silva, K. J., Brunings, A., Peres, N. A., Mou, Z., & Folta, K. M., (2015). The *Arabidopsis* NPR1 gene confers broad-spectrum disease resistance in strawberry. *Transgenic Res.*, 24(4), 693–704.
- Singh, A., (2015). Soil salinization and waterlogging: A threat to environment and agricultural sustainability. *Ecol. Indic.*, 57, 128–130.
- Singh, A., (2018). Salinization of agricultural lands due to poor drainage: A viewpoint. *Ecol. Indic.*, 95, 127–130.
- Singh, S., Singh, A., Kumar, S., Mittal, P., & Singh, I. K., (2018). Protease inhibitors: Recent advancement in its usage as a potential biocontrol agent for insect pest management. *Insect Sci.* <https://doi.org/10.1111/1744-7917.12641>.
- Sripriya, R., Parameswari, C., & Veluthambi, K., (2017). Enhancement of sheath blight tolerance in transgenic rice by combined expression of tobacco osmotin (ap24) and rice chitinase (chi11) genes. *Vitro Cell Dev Biol. Plant.*, 53(1), 12–21.
- Su, Y., Wang, Z., Liu, F., Li, Z., Peng, Q., Guo, J., Xu, L., & Que, Y., (2016). Isolation and characterization of ScGluD2, a new sugarcane beta-1, 3-glucanase D family gene induced by *Sporisorium scitamineum*, ABA, H<sub>2</sub>O<sub>2</sub>, NaCl, and CdCl<sub>2</sub> stresses. *Front Plant Sci.*, 7, 1348.
- Su, Y., Xu, L., Wang, S., Wang, Z., Yang, Y., Chen, Y., & Que, Y., (2015). Identification, phylogeny, and transcript of chitinase family genes in sugarcane. *Sci. Rep.*, 5, 10708.
- Takemoto, D., & Mizuno, Y., (2016). Belowground and aboveground strategies of plant resistance against *Phytophthora* species. In: *Belowground Defense Strategies in Plants* (pp. 151–169). Springer, Cham.
- Thakur, A. K., Singh, K. H., Sharma, D., Singh, L., Parmar, N., Nanjundan, J., & Khan, Y. J., (2018). Transgenic development for biotic and abiotic stress management in horticultural crops. In: *Genetic Engineering of Horticultural Crops* (pp. 353–386). Academic Press, London.
- Tigchelaar, M., Battisti, D. S., Naylor, R. L., & Ray, D. K., (2018). Future warming increases the probability of globally synchronized maize production shocks. *Proc. Natl. Acad. Sci.*, 115, 6644–6649. doi: 10.1073/pnas.1718031115.
- Todaka, D., Shinozaki, K., & Yamaguchi-Shinozaki, K., (2015). Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Front Plant Sci.*, 6, 84.
- Trębicki, P., Nancarrow, N., Cole, E., Bosque-Pérez, N. A., Constable, F. E., Freeman, A. J., Rodoni, B., et al., (2015). Virus disease in wheat is predicted to increase with a changing climate. *Glob. Chang. Biol.*, 21(9), 3511–3519.
- Ullah, A., Hussain, A., Shaban, M., Khan, A. H., Alariqi, M., Gul, S., Jun, Z., et al., (2018). Osmotin: A plant defense tool against biotic and abiotic stresses. *Plant Physiol. Biochem.*, 123, 149–159.
- Vannini, C., Campa, M., Iriti, M., Genga, A., Faoro, F., Carravieri, S., Rotino, G. L., et al., (2007). Evaluation of transgenic tomato plants ectopically expressing the rice Osmyb4 gene. *Plant Science*, 173, 231–239.
- Vannini, C., Locatelli, F., Bracale, M., Magnani, E., Marsoni, M., Osnato, M., Mattana, M., et al., (2004). Overexpression of the rice Osmyb4 gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. *The Plant Journal*, 37, 115–127.
- Vaughan, M. M., Block, A., Christensen, S. A., Allen, L. H., & Schmelz, E. A., (2018). The effects of climate change associated abiotic stresses on maize phytochemical defenses. *Phytochem. Rev.*, 17, 37–49. doi: 10.1007/s11101-017-9508-2.

- Wan, Q., Hongbo, S., Zhaolong, X., Jia, L., Dayong, Z., & Yihong, H., (2017). Salinity tolerance mechanism of osmotin and osmotin-like proteins: A promising candidate for enhancing plant salt tolerance. *Curr. Genomics*, 18(6), 553–556.
- Wang, F. W., Wang, M. L., Guo, C., Wang, N., Li, X. W., Chen, H., Dong, Y. Y., et al., (2016a). Cloning and characterization of a novel betaine aldehyde dehydrogenase gene from *Suaeda corniculata*. *Genet. Mol. Res.*, 15, 15027848.
- Wang, H., Wang, H., Shao, H., & Tang, X., (2016b). Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. *Front Plant Sci.*, 7, 67.
- Wang, H., Zou, Z., Wang, S., & Gong, M., (2013). Global analysis of transcriptome responses and gene expression profiles to cold stress of *Jatropha curcas* L. *PLoS One*, 8, e82817. doi: 10.1371/journal.pone.0082817.
- Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., & Gao, C., (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat. Biotechnol.*, 32, 947–951. doi: 10.1038/nbt.2969.
- Wani, S. H., Sah, S. K., Hossain, M. A., Kumar, V., & Balachandran, S. M., (2016). Transgenic approaches for abiotic stress tolerance in crop plants. In: *Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits* (pp. 345–396). Springer, Cham.
- Waqas, M. A., Khan, I., Akhter, M. J., Noor, M. A., & Ashraf, U., (2017). Exogenous application of plant growth regulators (PGRs) induces chilling tolerance in short-duration hybrid maize. *Environ. Sci. Pollut. Res.*, 24, 11459–11471. doi: 10.1007/s11356-017-8768-0.
- Xia, N., Zhang, G., Liu, X. Y., Deng, L., Cai, G. L., Zhang, Y., Wang, X. J., Zhao, J., Huang, L. L., & Kang, Z. S., (2010). Characterization of a novel wheat NAC transcription factor gene involved in defense response against stripe rust pathogen infection and abiotic stresses. *Molecular Biology Reports*, 37, 3703–3712.
- Zafar, S. A., Noor, M. A., Waqas, M. A., Wang, X., Shaheen, T., & Raza, M., (2018). Temperature extremes in cotton production and mitigation strategies. In: *Past, Present, and Future Trends in Cotton Breeding* (London, UK: IntechOpen). doi: 10.5772/intechopen.74648.
- Zandalinas, S. I., Mittler, R., Balfagón, D., Arbona, V., & Gómez-Cadenas, A., (2018). Plant adaptations to the combination of drought and high temperatures. *Physiol. Plant.*, 162(1), 2–12.
- Zhang, H. Y., Liu, H. M., & Liu, X. Z., (2015b). Production of transgenic kiwifruit plants harboring the SbtCry1Ac gene. *Genet. Mol. Res.*, 14, 8483–8489.
- Zhang, L., Davies, L. J., & Elling, A. A., (2015a). A Meloidogyne incognita effector is imported into the nucleus and exhibits transcriptional activation activity in planta. *Mol. Plant Pathol.*, 16(1), 48–60.
- Zhang, L., Zhao, H. K., Dong, Q. L., Zhang, Y. Y., Wang, Y. M., Li, H. Y., Xing, G. J., et al., (2015c). Genome-wide analysis and expression profiling under heat and drought treatments of HSP70 gene family in soybean (*Glycine max* L.). *Front Plant Sci.*, 6, 773.
- Zhu, J. K., (2016). Abiotic stress signaling and responses in plants. *Cell*, 167(2), 313–324.
- Zörb, C., Geilfus, C. M., & Dietz, K. J., (2019). Salinity and crop yield. *Plant Biol.*, 21, 31–38. doi: 10.1111/plb.12884.

## CHAPTER 7

---

# Towards Engineering Smart Transcription Factors for Enhanced Abiotic Stress Tolerance in Plants

SYED SARFRAZ HUSSAIN,<sup>1</sup> BUSHRA RASHEED,<sup>2</sup> ABDUL QAYYUM RAO,<sup>2</sup> AHMAD ALI SHAHID,<sup>2</sup> and BUJUN SHI<sup>3</sup>

<sup>1</sup>*Kauser Abdulla Malik School of Life Sciences (KAM-SLS),  
Forman Christian College (A Chartered University), Ferozepur Road,  
Lahore – 54600, Pakistan*

<sup>2</sup>*National Center of Excellence in Molecular Biology (NCEMB),  
West Canal Bank Road, Thokar Niaz Baig, University of The Punjab,  
Lahore, Pakistan*

<sup>3</sup>*School of Agriculture, Food, and Wine, University of Adelaide, SA – 5064,  
Australia*

*\*Corresponding author. E-mail: syedsarfraz\_2000@yahoo.com*

---

## ABSTRACT

Environmental stresses (both biotic and abiotic stresses) greatly affected plant productivity. Drought and associated stresses pose a major hindrance to plant growth and productivity around the globe because of the complex nature involving multigene, which further complicates the genetic engineering of crops. Ultimately, these genes either provide stress protection to plants or perform important regulatory functions. Because drought stress tolerance in plants is triggered by complex multicomponent signally pathways therefore, a basic concept of gene regulatory networks along with physiological and biochemical understanding is essential for plant engineering with high-stress

---

Biology and Biotechnology of Environmental Stress Tolerance in Plants, Volume 3: Sustainable Approaches for Enhancing Environmental Stress Tolerance. Aryadeep Roychoudhury (Ed.)

© 2024 Apple Academic Press, Inc. Co-published with CRC Press (Taylor & Francis)

tolerance. As opposed to structural genes, TFs are complex proteins and control multiple steps in biochemical pathways leading to stress tolerance. Therefore, TFs have emerged as promising tools for the manipulation of complex stress-responsive regulatory pathways in plants. Recently, the functions of a number of plant TFs have been investigated in drought and associated stresses, which may lead to devising powerful and workable strategies for engineering transgenic plants with enhanced tolerance. As a result of collection of huge data on the involvement of plant bZIP, HD-Zip, DREB/CBF, WRKY, and MYB TFs in the regulation of stress responses including drought, discussion in this chapter move around these TFs. Prospects of commercial manipulation of rationally designed TFs and CRISPR-Cas9 based TFs manipulations in agricultural biotechnology along with interactions among TFs within stress responsive networks is discussed. The main objective of this chapter is to discuss approaches for engineering improved stress tolerance of crop plants using naturally existing as well as synthetic/tailored variants of TFs for enhanced abiotic stress tolerance.

## **7.1 INTRODUCTION**

Abiotic stresses are known causes of significant yield loss of crop plants around the globe, leading to an average yield loss of over 50% for most major crops (Hussain et al., 2011a; Hu & Xiong, 2014; Khoshmanzar et al., 2019). Drought stress alone have the potential to reduce crop yield by more than 50% when it occurs at reproductive stage of crop plants (Valliyodan & Nguyen, 2006; Langridge et al., 2006; Hussain et al., 2012; Guttikonda et al., 2014). Abiotic stresses such as drought and associated stresses have negative impact on several vital crop traits (Aroca et al., 2012; Calvo-Polanco et al., 2012; Theocharis et al., 2012; Shrivastava et al., 2015; Hoang et al., 2017) including normal growth and development, photosynthetic capacity, biomass accumulation, and plant yield (Fleury et al., 2010; Kreuzweiser & Rennenberg, 2014; Asgari Lajayer et al., 2017), hence leading to global food insecurity. Plant breeders have made immense progress in generating and improving abiotic stress-tolerant crop plant, but still serious efforts are required because plant productivity and crop yield fail to meet global food demands in the face of harsh weather conditions, climate change scenarios and ever-increasing world population (Hu & Xiong, 2014; Baillo et al., 2019). Considering that the yield of important cereal crops such as maize, rice, wheat, barley, and sorghum could not be increased in near future (Ray

et al., 2012), this will force plant researchers to think about integrated and sustainable functional approaches. Furthermore, narrow genetic base due to absence of natural variation in plant breeding stock has led to greater sensitivity to different abiotic stresses (Hussain et al., 2012; Lobell et al., 2014; Khan et al., 2019). Moreover, multiple genes control the tolerance to various abiotic stresses, thereby further increasing the complexity for enhancing the stress tolerance of major commercial crop cultivars and seems more challenging (Li et al., 2018). As part of the evolutionary process, plants have developed various strategies to combat unfavorable abiotic stresses do not seem effective in crop plants (Langridge et al., 2006; Hrmova & Lopato, 2014).

Classical approaches for improvement in plant performance under various abiotic stresses were not very successful because genes/alleles contributing to stress tolerance are mostly disappeared during the lengthy conventional breeding process. This is simple to understand that these alleles are lost because possibly these do not have a direct contribution to the yield of crop plants (Bartels & Hussain, 2008; Zheng et al., 2008). On the other hand, researchers have struggled hard to develop crop plants with enhanced stress tolerance using both conventional and transgenic approaches, literally no or little success has been achieved in the field mainly due to following reasons: (i) fundamental molecular mechanisms of stress tolerance are not yet fully understood; (ii) complex multigene nature of stress tolerance; and (iii) lack of data on different stressors interaction (Hussain et al., 2011a, 2012). Therefore, scientists around the globe are reluctant to employ classical approaches which are less efficient, and labor intensive and time consuming (Ashraf & Foolad, 2007; Hussain et al., 2012).

Progress in understanding of gene expression, signal transduction and transcriptional regulation in plants responses to environmental constraints is exceptional. Similarly, gene discovery has been greatly facilitated by recent advances in molecular, genomic, and other high throughput tools (Yamaguchi et al., 2007; Kitsios & Doonan, 2011; Zwack & Rashotte, 2015). Understanding of plant responses at cellular, molecular, metabolic, physiological, and genetic levels to abiotic stress conditions and development of approaches towards improving plant stress tolerance has facilitated gene revolution following green revolution (Hussain et al., 2011b; Cabello et al., 2014). These discoveries made available different functional or regulatory genes for genetic engineering for improving stress tolerance of crop plants (Valliyodan & Nguyen, 2006; Bhatnagar-Mathur et al., 2007; Kathuria et al., 2007; Hussain et al., 2011a, b, 2012; Hrmova & Lopato, 2014; Baillo et

al., 2019; Khan et al., 2019; Ahmed et al., 2020; Hrmova & Hussain, 2021). However, these genetic engineering approaches are flexible because they can aid in conventional breeding efforts (Capell et al., 2004).

In addition, development of novel high throughput techniques in molecular biology in combination with bioinformatic science have contributed to the emergence of system based high throughput tools such as genome editing tools, thus ensuring a comprehensive source of biological resources which transformed conventional plant breeding to precision plant breeding (Deikman et al., 2011; Weber & Fussenegger, 2011; Tardieu, 2012; Cabello et al., 2014). Importance of cis-trans engineering using genome editing tools and manipulation of synthetic/artificial transcription factors (ATFs) would be highlighted and discussed with emphasis on the role of such TFs as genetic switches in stress tolerance.

### **7.1.1 CONTRIBUTION OF CONVENTIONAL BREEDING FOR PLANT ABIOTIC STRESS TOLERANCE**

Development of high yielding and stress-tolerant genotypes is a long-standing goal of agricultural scientists, but conventional breeding has met with limited success in realization of this goal due to multigenic nature of tolerance traits (Richards, 1996; Hussain et al., 2012; Hrmova & Hussain, 2021). Another reason of this understanding is low heritability of the tolerance genes in crop plants (Fritsche-Neto & Borem, 2012). Generally, breeding schemes use different selection procedures which constitutes critical steps in the early selection of superior materials. For example, quantitative characters like environmental stress tolerance, selection strategy involve both stress and non-stress scenarios for selection. Generally breeding methods for quantitative characters such as abiotic stress tolerance, yield, and other economic traits are the same. Although conventional breeding has significantly contributed to tolerant crop lines development, specific phenotype identification with abiotic stress tolerance traits is still a big challenge for using this approach in other commercial crop plants. Wild relatives of grasses could be the main source of stress-related genes because these usually demonstrated high tolerance to various stresses. Therefore, there is the possibility that these desired traits can be transferred from wild relatives to domesticated plants. As a matter of fact, 10–20% of such variations have already been introgressed into modern tetraploid/hexaploidy wheat varieties (Langridge et al., 2006). Despite challenges, conventional breeding efforts have contributed towards the development of drought and heat tolerant crops. For example, Haley et al.

(2007) reported the development of drought tolerant wheat variety, Ripper. This variety has the potential to perform well under drought conditions with high yield and other desirable characteristics. Considering both biotic and abiotic stresses in variety development has far-reaching consequences. Stress tolerant maize development programs reported a number of maize lines with high productivity under various biotic and abiotic (drought) stresses (Badu-Apraku & Yallou, 2009).

Generation and availability of molecular tools, especially phenotypic, physiological, biochemical, and genetic markers, and molecular markers (specific gene or QTL associated markers) have contributed immensely towards the improvement of crop plants under abiotic stresses (Vinocur & Altman, 2005). Similarly, molecular markers have the potential to identify candidate genes with superior allelic variations in the breeding populations (Tahmasebi et al., 2017). Merchuk-Ovnat et al. (2016) used QTLs introgression between wild emmer wheat and commercial wheat. This breeding-based scheme improved drought tolerance in commercial tetraploid and hexaploid wheat. Similarly, recombinant inbred wheat population has been used for QTLs mapping for combined heat and drought stresses. This strategy has also demonstrated improved drought and heat individually or combination of both stresses (Tahmasebi et al., 2017).

Emerging high throughput technologies offer an alternative to classical breeding to identify and study specific loci with significant role in plant tolerance (Lamaoui et al., 2018). Recently, genomic tools along with DNA sequencing techniques and bioinformatics programs have revolutionized plant biotechnological research. Similarly, genetic engineering tools can also use to modify a biological function in transgenic plants (Hussain et al., 2012; Zandalinas et al., 2017). Therefore, genetic engineering schemes could help to manipulate desirable tolerance traits into crop plants (Hussain et al., 2012; Zandalinas et al., 2017). Over the past decade, several studies used genetic engineering as a tool for developing plants with enhanced abiotic stress tolerance and successfully achieved significantly improved abiotic stress tolerance in crop plants (Hussain et al., 2011b, 2012; Alvarez-Gerding et al., 2015; Nuccio et al., 2015; Corrales et al., 2017).

### **7.1.2 GENETIC ENGINEERING FOR ENHANCED ABIOTIC STRESS TOLERANCE**

A plethora of research has contributed to our understanding of main steps of gene expression, transcriptional regulation, and signal transduction in plant



responses to various abiotic stresses (Shinozaki et al., 2003; Yamaguchi-Shinozaki & Shinozaki, 2005). Similarly, high throughput genomic techniques in combination with bioinformatics analyzes have greatly facilitated gene function and cloning (Seki et al., 2001, 2007; Abe et al., 2003; Tran et al., 2004). These developments enabled manipulation of functional or regulatory genes for stress tolerance to various abiotic stresses in plants (Trujillo et al., 2009).

Transgenic approaches in developing tolerant plants to different abiotic stress have been significantly enhanced by one or multigene transfer to the target plant. Overexpression of target genes in model and crop plants allowed researchers to explore and validate the molecular mechanism of stress tolerance and their protective effects against various environmental stresses. After detailed research, it is concluded that two gene categories exist. Regulatory genes are either involved in signaling and/or regulatory pathways other genes encode enzymes resulting in the synthesis of functional and structural protectants (Bartels & Hussain, 2008; Hu et al., 2010; Hussain et al., 2012). Another development is that state of the art technologies allow to characterize many genes simultaneously to explore their structure, function, and interaction under stress. Though labor intensive, generating transgenic plants seems a tricky but suitable option for generating tolerant plants, given the multigenic nature of abiotic stress tolerance, potential negative effects on plant growth and other scientific limitations.

Metabolic engineering for higher compatible solutes/osmolyte (referred to as osmoprotectants) has shown remarkable success in different plants under various stresses (Garg et al., 2002; Wang et al., 2003; Park et al., 2007). However, Serraj & Sinclair (2002) raised questions on the real advantages of such plant engineering strategy. Several studies have successfully engineered crop plants and discussed the functions of the osmolytes in plants under stress (Hasegawa et al., 2000; Garg et al., 2002; Chaves & Oliveira, 2004; Vinocur & Altman, 2005; Valliyodan & Nguyen, 2006; Kumar et al., 2006; Molinari et al., 2007; Gubis et al., 2007; Park et al., 2007; Vendruscolo et al., 2007; Ahmad et al., 2008; Chen et al., 2009; Suarez et al., 2009; Alcazar et al., 2010; Thippeswamy et al., 2010). Plants use these osmolytes for maintaining turgor pressure, decreasing the osmotic potential in the cytoplasm, protects different cellular compartments from injury, and help to stabilize the structure and function of macromolecules (sensitive proteins and the vital membranes) under different environmental constraints (McNeil et al., 1999; Hasegawa et al., 2000; Park et al., 2007; Hussain et al., 2012). Several compatible solutes such as Glycine betaine (Naidu et al., 1991;

Wyn Jones, 1994; Papageorgiou & Murata, 1995; Nuccio et al., 1998, 1999; McNeil et al., 2000, 2001; Sakamoto & Murata, 2001), trehalose (Singer & Lindquist, 1998; Goddijn and van Dun, 1999; Yeo et al., 2000; Iturriaga et al., 2000; Karim et al., 2007; Paul et al., 2008), proline (Kishor et al., 1995; Yoshiba et al., 1997; Fujita et al., 1998; Kumar et al., 2003; Claussen, 2005; Chen et al., 2009), trigonelline (Nomura et al., 1995; McNeil et al., 1999, 2000, 2001; Nuccio et al., 1998, 1999) and sugar alcohol/polyols (Thomas et al., 1995; Bohnert & Jensen, 1996; Smart & Flores, 1997; Sheveleva et al., 1998; Rajam et al., 1998) have been characterized in plants under stress.

Based on the above discussion, it is easy to conclude that osmoprotectants accumulate in plants under stress is generally taken as a protective approach leading to plant survival in changing climate (Hussain et al., 2012). However, it is also known that several important crop plants do not synthesize or accumulate these osmolytes in high quantity under stress. Therefore, the generation of transgenic plants overexpressing specific osmolytes genes is widely adopted strategy around the globe. Transgenic manipulation of these genes serves as a suitable tool to raise crop plants with enhanced tolerance to various stresses. Metabolic engineering of osmolyte/osmoprotectant genes has got momentum several successful examples can be coined where different genes encoding osmoprotectants have been overexpressed, like glycine betaine (Su et al., 2006; Park et al., 2007; Ahmad et al., 2008; Yang et al., 2008; Zhou et al., 2008; Yu et al., 2009; Goel et al., 2011; Luo et al., 2012), proline (Kumar et al., 2010; Thippeswamy et al., 2010; Jazii et al., 2011; Karthikeyan et al., 2011; Behelgardy et al., 2012; Li et al., 2013; Mehboobeh & Akbar, 2013; Guerzoni et al., 2014; Liu et al., 2014; Shrestha et al., 2014; Reddy et al., 2015; Zhang et al., 2015; Guan et al., 2018, 2019; Wang et al., 2019), trehalose and sugar alcohols (Almeida et al., 2007; Karim et al., 2007; Stiller et al., 2008; Suzuki et al., 2008; Suárez et al., 2009; Krasensky et al., 2014; Wang et al., 2020). Vast data have highlighted the importance of different osmolytes/compatible solutes/osmoprotectants in plant tolerance to various stresses and successfully demonstrated that genetic manipulation for accumulation of these compounds has potential applications in developing stress-tolerant plants (Alzahrani, 2021).

Major stress-associated proteins include heat shock proteins (HSPs), late embryogenesis abundant (LEA)-type proteins, and cold shock-domain family proteins (CSPs) accumulate during various abiotic stresses such as drought, high salt, oxidative stress, and heat stress (Volkov et al., 2006; Sato & Yokoya, 2008; Jiang et al., 2009; Jyothsnakumari et al., 2009; Liu et al., 2010). These proteins function as molecular chaperones, which are involved

in protection of the cellular machinery during different cellular processes (Vierling, 1991; Hendrick & Hartl, 1993; Boston et al., 1996; Hartl, 1996; Waters et al., 1996; Török et al., 2001). Similar to HSPs, LEA-type proteins have been identified and characterized in diverse plant species where these are responsible for conferring tolerance to various abiotic stresses in plants (Kasuga et al., 1999; Shih et al., 2004; Grelet et al., 2005; Jyothsnakumari et al., 2009). In 2010, a group of researchers floated the idea of multifunctional gene (Hu et al., 2010). A multifunctional gene is a specific gene that can simultaneously control several kinds of traits and effectively manifest several different types of functions. Based on the above concept, LEA, sHSPs, and other proteins perform multiple functions in plants under stress. Therefore, plant tolerance to stresses can be developed by transforming plants with multifunctional genes (Hussain et al., 2011b).

Transcription factors (TFs) are one of the major genetic circuits in the gene regulatory pathways underlying plant responses to abiotic stresses (Baldoni et al., 2015; Hrmova & Hussain, 2021). TFs are responsible for regulation of transcriptional expressions of stress-related genes by binding specifically to cis-elements in the promoter regions of target genes, thereby regulating stress tolerance/resistance of plants under abiotic stresses (Baldoni et al., 2015; Hoang et al., 2017; Hrmova & Hussain, 2021). Based on differences in DNA-binding domains, TFs have been divided into 64 families such as NAC, WRKY, MYB, HD-ZIP, bZIP, EREBP/AP2 (Hoang et al., 2017). A plethora of research has shown that these TFs families have a central role in defining plant tolerance to abiotic stresses via abscisic acid (ABA) dependent and independent pathways (Yamaguchi-Shinozaki & Shinozaki, 2005, 2006; Pandey & Somssich, 2009; Dubos et al., 2010; Chen et al., 2012; Nuruzzaman et al., 2013). Overexpression of TFs have resulted in abiotic stress tolerance in plants, thereby promising candidates for enhancing stress tolerance in crop plants via genetic engineering.

## **7.2 TRANSCRIPTION FACTORS IN ABIOTIC STRESS**

Plants being sessile, have developed clever defense mechanisms to abiotic stresses such as interconnected genetic networks and circuits which are mainly managed by highly sensitive signal transduction cascades (Hrmova & Hussain, 2021). Several studies have highlighted the central role of TFs in gene regulation and expression which is known as transcriptional control (Kang et al., 2002; Bartels & Sunkar, 2005; Hussain et al., 2011a; Wang et

al., 2016; Khan et al., 2018). The importance of TFs is also evident from the fact that around 10% of completely sequenced plant genomes encode TFs (Baillo et al., 2019). Sequencing data have also reported the presence of 3,337, 1,611, 2,450, 1,922 TFs in maize, rice, sorghum, and Arabidopsis, respectively (Wang et al., 2011; Hoang et al., 2017; Baillo et al., 2019; Hrmova & Hussain, 2021). Therefore, specific TFs in functional states may be needed in different biological systems including abiotic stresses. Similarly, Inukai et al. (2017) provided a detailed description of systematic engagement of TFs and their interactions during gene regulation and expression. Besides multigenic in nature, stress tolerance is one of complex plant trait and TFs have the potential to regulate simultaneously several stress responsive downstream in plants (Mittler & Blumwald, 2010; Hussain et al., 2011a; Atkinson & Urwin, 2012).

Transcription factors are central regulatory proteins that underpins the regulation of plant gene by binding to the cis-element of a specific gene and control critical aspects of gene function such as stress tolerance, ensuring normal growth and development of plants under extreme stresses (Bartels & Sunkar, 2005; Hrmova & Lopato, 2014). Several TFs have been identified in plants and common TFs involved in stress mitigation in plants include the APETALA2/ethylene response factor proteins (Hinz et al., 2010; Quan et al., 2010; Liu et al., 2012; Schmidt et al., 2013; Vogel et al., 2014; Zhu et al., 2014), NAC (De Clercq et al., 2013; Singh et al., 2013; Van Ha et al., 2014; Fang et al., 2015), basic region leucine zipper motifs (Kang et al., 2002; Fujita et al., 2005; Furihata et al., 2006; Hartmann et al., 2015), WRKY (Seo et al., 2012; Jiang et al., 2014; Huang et al., 2015; Diao et al., 2016; He et al., 2016), MYB (Meng et al., 2014, 2015; Sun et al., 2014; Cai et al., 2015), NAC (NAM, CUC2, ATAF1/2), and homeodomain-leucine zipper protein families (Xiang et al., 2008; Fujita et al., 2011; Huang et al., 2012; Puranik et al., 2012; Todaka et al., 2012; Ehong et al., 2016; Liu et al., 2018; Mao et al., 2019; Yu et al., 2021; Ma et al., 2021). Several crop and model plants overexpressing different TFs demonstrated enhanced tolerance to different abiotic stresses due to expression of many stress-responsive genes (Xiong et al., 2014; Alvarez-Gerding et al., 2015; Jisha et al., 2015; Zhang et al., 2015; Zhu et al., 2015; Liu et al., 2016; Rahman et al., 2016; Yu et al., 2021). Taken together, these findings suggest that genetic engineering of plants using different TFs genes has the potential to stimulate general or specific pathways related to abiotic responses, leading to improved plant tolerance to various abiotic stresses (Table 7.1).

**TABLE 7.1** Transcription Factor Overexpression Lead to Enhanced Abiotic Stress Tolerance in Plants

TF	Gene	Transgenic Plant	Effect	References
NAC	PbeNAC1	Tobacco	↑ Cold/Drought tolerance	Wang et al. (2016)
	JUB1	Arabidopsis	↑ Dehydration tolerance	Ebrahimian-Motlagh et al. (2017)
	ANAC069	Arabidopsis	↑ Salt/osmotic tolerance	He et al. (2016)
	GsNAC019	Arabidopsis	↑ Alkaline tolerance	Cao et al. (2017)
	ONAC022	Rice	↑ Salt/drought tolerance	Hong et al. (2016)
	TaRNAC1	Wheat	↑ Drought tolerance	Chen et al. (2018)
	TaNAC47	Arabidopsis	↑ Salt/drought/freezing tolerance	Zhang et al. (2016)
	MfNACsa	Medicago	↑ Drought tolerance	Duan et al. (2017)
	SINAC4	Tomato	↑ Drought/salt tolerance	Zhu et al. (2014)
	SINAC35	Tobacco	↑ Drought/salt tolerance	Wang et al. (2016)
	ZmNAC55	Arabidopsis	↑ Drought tolerance	Mao et al. (2016)
	TaNAC2L	Arabidopsis	↑ Heat tolerance	Guo et al. (2015)
	SINAM1	Tobacco	↑ Cold tolerance	Li et al. (2016)
	NAC26	Arabidopsis	↑ Drought tolerance	Fang et al. (2016)
	MbNAC25	Arabidopsis	↑ Salinity, cold tolerance	Han et al. (2020)
	MdNAC1	Apple	↑ Drought tolerance	Jia et al. (2019)
	MINAC10	Arabidopsis	↑ Drought, salinity tolerance	He et al. (2019)
	MbNAC25	Arabidopsis	↑ Cold, salinity tolerance	Han et al. (2020)
	MINAC12	Arabidopsis	↑ Salt, drought tolerance	Yang et al. (2018)
	AemNAC2, AemNAC3	Wheat	↑ Cadmium tolerance	Du et al. (2020)
	AhNAC4	Tobacco	↑ Drought tolerance	Tang et al. (2017)
	CarNAC6	Arabidopsis	↑ Dehydration tolerance	Liu et al. (2017)
	GmNAC019	Arabidopsis	↑ Drought tolerance	Hoang et al. (2019)
	GmNAC109	Arabidopsis	↑ Drought, salt	Yang et al. (2019)
	GmSNAC49	Arabidopsis	↑ Drought tolerance	So & Lee (2019)
	OsNAC14	Rice	↑ Drought tolerance	Shim et al. (2018)
	OsNAC5, OsNAC6, OsNAC9, OsNAC10	Rice	↑ Drought tolerance	Chung et al. (2018)
	PgNAC21	Arabidopsis	↑ Salinity tolerance	Shinde et al. (2019)
	TaRNAC1	Wheat	↑ Drought tolerance	Chen et al. (2018)
	ONAC066	Rice	↑ Drought and oxidative tolerance	Yuan et al. (2019)

**TABLE 7.1** (Continued)

TF	Gene	Transgenic Plant	Effect	References
bZIP	BoNAC019	Arabidopsis	↓ Drought tolerance	Wang et al. (2018)
	JUNGBRUNNEN 1	Tomato	↑ Drought tolerance	Thirumalaikumar et al. (2018)
	GmTGA15	Soybean, arabidopsis	↑ Drought tolerance	Chen et al. (2021)
	AtABF3	Alfalfa	↑ Salt/drought tolerance	Wang et al. (2016)
	OsABF1	Rice	↑ Drought tolerance	Zhang et al. (2016)
	OsbZIP46	Rice	↑ Drought/high temperature tolerance	Chang et al. (2017)
	TaABL1	Arabidopsis	↑ Salt/drought/low temperature tolerance	Xu et al. (2014)
	TabZIP6	Arabidopsis	↑ Freezing tolerance	Cai et al. (2018)
	TabZIP60	Arabidopsis	↑ Salt/drought/low temperature tolerance	Zhang et al. (2015)
	ABP9	Cotton	↑ Drought/salt tolerance	Wang et al. (2017)
	SlbZIP38	Tomato	↑ Drought/salt tolerance	Pan et al. (2017)
	EcbZIP60	Tobacco	↑ Drought tolerance	Babitha et al. (2015)
	GmbZIP110	Arabidopsis	↑ Salinity tolerance	Xu et al. (2016)
	GmFDL19	Soybean	↑ Drought/salt tolerance	Li et al. (2017)
	SlbZIP1	Tomato	↑ Drought, salt tolerance	Zhu et al. (2018)
	OsbZIP16	Arabidopsis, rice	↑ Drought tolerance	Pandey et al. (2018)
	CaBZ1	Potato	↑ Drought tolerance	Moon et al. (2015)
	GhABF2	Arabidopsis	↑ Drought tolerance	Liang et al. (2016)
	BnbZIP2	Arabidopsis	↑ Salt/drought tolerance	Huang et al. (2016)
	GhABF2	Arabidopsis	↑ Drought tolerance	Liang et al. (2016)
	GhABF2	Cotton	↑ Drought tolerance	Liang et al. (2016)
	IbABF4	Sweet potato	↑ Drought, salt, cold tolerance	Wang et al. (2019)
	IbABF4	Arabidopsis	↑ Drought, salt, cold tolerance	Wang et al. (2019)
	IbbZIP1	Arabidopsis	↑ Salt, drought tolerance	Kang et al. (2019)
	OsbZIP6	Rice	↑ Drought, oxidative tolerance	Yang et al. (2019)
	SlbZIP38	Tomato	↑ Drought tolerance	Pan et al. (2017)
	AtTGA4	Arabidopsis	↑ Drought tolerance	Zhong et al. (2015)
MYB	FtMYB9	Arabidopsis	↑ Salt/drought tolerance	Gao et al. (2017)

**TABLE 7.1** (Continued)

TF	Gene	Transgenic Plant	Effect	References
	IR-MYB	Chickpea	↑ Drought tolerance	Ramalingam et al. (2015)
	OsMYB6	Rice	↑ Drought tolerance	Tang et al. (2019)
	TaMYBsm3	Arabidopsis	↑ Drought tolerance	Li et al. (2019)
	TaMpc1-D4	Wheat	↑ Drought tolerance	Li et al. (2020)
	HvMYB1	Barley	↑ Drought tolerance	Alexander et al. (2019)
	PtrMYB94	Arabidopsis	↑ Drought tolerance	Fang et al. (2020)
	MYB96	Arabidopsis	↑ Cold tolerance	Lee & Seo (2015)
	GmMYB84	Soybean	↑ Drought tolerance	Wang et al. (2017)
	GmMYB12B2	Arabidopsis	↑ Salt tolerance	Li et al. (2016)
	OsMYb55	Maize	↑ Drought tolerance	Casaretto et al. (2016)
	OsMYBc	Rice	↑ Salt	Wang et al. (2015)
	MID1	Rice	↑ Drought	Guo et al. (2016)
	OsMYB91	Rice	↑ Salt	Zhu et al. (2015)
	TaSIM	Arabidopsis	↑ Salt	Yu et al. (2017)
	MtMYBS1	Arabidopsis	↑ Salinity	Zhang et al. (2016)
	ARS1	Tomato	↑ Salt	Campos et al. (2016)
	GaMYB62L	Arabidopsis	↑ Drought	Butt et al. (2017a)
	GaMYB85	Arabidopsis	↑ Drought	Butt et al. (2017b)
	MdMYB88/ MdMYB124	Arabidopsis and apple	↑ Cold	Xie et al. (2018)
	PacMYBA	Arabidopsis	↑ Salt	Shen et al. (2017)
	AtMYB12	Arabidopsis	↑ Drought	Wang et al. (2016)
	AtMYB94	Arabidopsis	↑ Dehydration/drought	Lee et al. (2015)
	AtMYB94/AtMYB96	Arabidopsis	↑ Dehydration/drought	Lee et al. (2016)
	AtMYB96	Arabidopsis	↑ Drought, freezing	Guo et al. (2013)
	AtMYB37	Arabidopsis	↑ Drought	Yu et al. (2016)
	AtMYB49	Arabidopsis	↑ Salt	Zhang et al. (2020)
	AtMYB20	Arabidopsis	↑ Salt	Cui et al. (2013)
	AtMYB42	Arabidopsis	↑ Salt	Sun et al. (2020)
	AtMYB30	Arabidopsis	↑ Salt	Gong et al. (2020)
	AtMYB96	Arabidopsis	↑ Cold	Lee et al. (2015)
	AtMYB30	Arabidopsis	↑ Heat, oxidative	Liao et al. (2017)
	RmMYB108	Arabidopsis	↑ Cold	Dong et al. (2021)
	BnMYB2	Arabidopsis	↑ Cadmium tolerance	Zhu et al. (2020)

**TABLE 7.1** (Continued)

TF	Gene	Transgenic Plant	Effect	References
WRKY	ZmWRKY65	Arabidopsis	↑ Drought, salt	Huo et al. (2021)
	OsWRKY47	Rice	↑ Drought	Raineri et al. (2015)
	WRKY71	Arabidopsis	↑ Salt	Yu et al. (2018)
	AtWRKY53	Arabidopsis	↑ Drought	Sun & Yu (2015)
	ZmWRKY17	Arabidopsis	↑ Drought	Cai et al. (2017)
	OsWRKY71	Rice	↑ Cold	Kim et al. (2016)
	TaWRKY146	Arabidopsis	↑ Drought	Ma et al. (2017)
	GmWRKY27	Soybean	↑ Salt/drought	Wang et al. (2015)
	SlWRKY3	Tomato	↑ Salt	Hichri et al. (2017)
	MtWRKY76	Medicago	↑ Drought/salt	Liu et al. (2017)
	GhWRKY6-like	Arabidopsis	↑ Salt	Ullah et al. (2017)
	RtWRKY1	Arabidopsis	↑ Salt	Du et al. (2017)
	GhWRKY33	Arabidopsis	↑ Drought	Wang et al. (2019)
	AtWRKY53	Arabidopsis	↑ Drought, salt	Zheng et al. (2020)
	AtWRKY57	Arabidopsis	↑ Drought	Jiang et al. (2012)
	GhWRKY21	Cotton	↑ Drought	Wang et al. (2020)
	GhWRKY25	Cotton	↑ Drought	Liu et al. (2015)
	CaWRKY40	Pepper	↑ Heat	Dang et al. (2013)
	BdWRKY36	<i>Brachypodium distachyon</i>	↑ Drought	Sun et al. (2014)
	FcWRKY70	<i>Fortunella crassifolia</i>	↑ Salt	Gong et al. (2015)
	OsWRKY74	Rice	↑ Pi starvation, cold	Dai et al. (2016)
	OsWRKY76	Rice	↑ Cold	Yokotani et al. (2013)
	GmWRKY17	Soybean	↑ Salt	Yan et al. (2014)
	JrWRKY2/7	<i>Juglans regia</i>	↑ Drought, cold	Yang et al. (2017)
	SbWRKY30	Sorghum	↑ Salt, drought	Yang et al. (2020)
	IbWRKY47	Sweet potato	↑ Salt	Qin et al. (2020)
	IbWRKY2	Sweet potato	↑ Salt, drought	Zhu et al. (2020)
	MdWRKY30	Apple	↑ Salt, osmotic stress	Dong et al. (2020)
	GbWRKY1	<i>Gossypium barbadense</i>	↑ Salt	Luo et al. (2019)
	VbWRKY32	<i>Verbena bonariensis</i>	↑ Cold	Wang et al. (2020)
	PgWRKY33/62	Pearl millet	↑ Salt, drought	Chanwala et al. (2020)
	PagWRKY75	Poplar	↑ Drought	Zhao et al. (2019)



**TABLE 7.1** (Continued)

TF	Gene	Transgenic Plant	Effect	References
bHLH	AtbHLH68	Arabidopsis	↑ Drought	Le et al. (2017)
	PebHLH35	Arabidopsis	↑ Drought	Dong et al. (2014)
	ZmPTF1	Maize	↑ Drought	Li et al. (2019)
	AtbHLH112	Arabidopsis	↑ Drought/salt	Liu et al. (2015)
	BcICE1	Canola	↑ Cold	Zhang et al. (2018)
	OsbHLH068	Arabidopsis	↑ Salt	Chen et al. (2017)
	TabHLH39	Arabidopsis	↑ Salt/drought/cold	Zhai et al. (2016)
	FtbHLH2	Arabidopsis	↑ Cold	Yao et al. (2018)
	FtbHLH2	Arabidopsis	↑ Cold	Yao et al. (2018)
	FtbHLH3	Arabidopsis	↑ Drought/oxidative	Yao et al. (2017)
	AH	Tomato	↑ Low temperature	Qiu et al. (2016)
	DIICE1	Arabidopsis	↑ Cold	Yang et al. (2019)
	PubHLH1	Tobacco	↑ Cold	Jin et al. (2016)
	ThbHLH1	Arabidopsis	↑ Osmotic stress	Ji et al. (2016)
	ZmPTF3	Maize	↑ Drought	Gao et al. (2018)
	GmbHLH57	Soybean	↑ Fe deficiency	Li et al. (2018)
	TabHLH1	Tobacco	↑ Pi/N starvation	Yang et al. (2016)
	OsbHLH035	Rice	↑ Salt	Chen et al. (2018)
	PtFIT	Populus	↑ Iron	Huang & Daim (2015)
	ILR3	Arabidopsis	↑ Iron	Samira et al. (2018)
	TabHLH49	Wheat	↑ Drought	Liu et al. (2020)
	AtbHLH68	Arabidopsis	↑ Drought	Le et al. (2017)
	AtbHLH18	Arabidopsis	↑ Fe deficiency	Cui et al. (2018)

Transcription factors constitute an integral part of the genetic circuits which dynamically regulate gene networks to enhance plant stress tolerance. DNA-binding domains (DBD) of plant TFs can be replaced with engineered zinc fingers or TAL effectors to meet specific needs (Beerli & Barbas, 2002; Townsend et al., 2009). Consequently, engineering DBD strategy of the TFs facilitate binding of TFs to any target genomic sequence in plants (Gitzinger et al., 2009; Lu et al., 2009). Synthetic/Artificial TFs (ATFs) are promising alternative to naturally occurring TFs and composed of a translational fusion of DBD and effector domains (ED: repressor or activator). ATFs have DNA binding specificity and potential to act on particular promoter to carryout activation or repression of a specific gene. Previously genome editing tools (ZF domains and TALEs) have been extensively used to design DBDs for ATFs.

However, development of novel high throughput tools such as CRISPR/Cas9 has provided vast possibilities to simultaneously manipulate several regulatory genes in a genome to introduce the tailored genetic switches in the plants for stress tolerance (Bagdanove & Voytas, 2011; Bortesi & Fischer, 2015).

### **7.2.1 TRANSCRIPTION FACTORS INVOLVED IN SIGNALING NETWORKS UNDER ABIOTIC STRESSES**

Signaling pathway in plants composed of three main steps viz Signal perception and transduction, and expression of several stress-responsive downstream genes, which in turn activate physiological, biochemical, and molecular adaptive responses (Huang et al., 2012; Hrmova & Lopato, 2014). Environmental stimuli-caused signals are recognized by different transcellular membranes which are present in cell wall or membrane including  $\text{Ca}^{2+}$  channels and  $\text{Ca}^{2+}$  binding proteins, histidine kinases, and G-protein coupled receptors (Misra et al., 2007; Schaller et al., 2008; Thapa et al., 2011; Boudsocq & Sheen, 2013; Hrmova & Lopato, 2014). Signal transductions occur through conversion of extracellular signals into intracellular ones through de novo synthesis of specific second messengers such as ROS, inositol phosphate, diacylglycerols, hexaphosphate. Stress signals are transduced to subsequent components in the signaling pathway towards the nucleus (Baxter et al., 2014; Zhu, 2016). Signaling molecules such as protein kinases, phosphatases, and others play central role in signal transduction under abiotic stresses in plants. These signaling molecules (activated kinases and phosphatases) initiate phosphorylation/dephosphorylation of TFs proteins (Danquah et al., 2014; Kudla et al., 2018; Noman et al., 2021). As a result of phosphorylation/dephosphorylation cascade, TFs lead to the expression of several stress-responsive downstream genes (Danquah et al., 2014; Noman et al., 2021). However, differential expression of stress-responsive genes under specific stress led to different transcription pattern compared to non-stressed control (Kulda et al., 2018). Meanwhile, accumulation of various stress-related proteins and molecules occur in vegetative tissues because of expression of various structural genes which lead to stress tolerance (Bhargava & Sawant, 2013; Schulz et al., 2013; Hrmova & Lopato, 2014; Ho et al., 2015). Complete pathway consists of complex regulatory circuits to manipulate the several stress-responsive downstream genes resulting in plant acclimation, survival under unfavorable environmental conditions (Ishihama & Yoshioka,

2012; Baillo et al., 2019; Hrmova & Hussain, 2021). In conclusion, engineering suitable TFs could potentially alleviate stress responses because they regulate the expression of several stress-responsive downstream genes by driving the specific regulatory elements in their promoter region (Hoang et al., 2017; Baillo et al., 2019).

Similarly, phytohormones also have significant contribution in normal growth and development of plants. ABA is one such hormone which play significant role in plant growth and development especially under various biotic and abiotic stresses (Fujita et al., 2013, 2014; Hrmova & Lopato, 2014; Hoang et al., 2019; Hrmova & Hussain, 2021). Generally, ABA is a stress-responsive because it is involved in conferring adaptive responses under environmental stresses (Bauer et al., 2013; Suzuki et al., 2013). Research data have reported various Interactions between ABA and different plant TFs under stress (Shang et al., 2010; Shan et al., 2012). Over 200 TFs of 20 gene families have been simultaneously characterized at a single developmental stage regulated by ABA in a study (Nemhauser et al., 2006). Based on reports, several drought-responsive genes show response to ABA and many genes are non-responsive to ABA. Therefore, plant stress tolerance is governed by either ABA-dependent or ABA-independent pathways (Yan et al., 2013; Wishwakarma et al., 2017; Baillo et al., 2019; Khan et al., 2019; Hrmova & Hussain, 2021). However, it is indicated that potentially huge gap exists in our understanding of stress-regulated (ABA-dependent and ABA-independent) signaling pathways under different abiotic stresses. However, TFs involved in ABA-dependent pathways could potentially be the natural target for genetic engineering to enhance abiotic stress tolerance of crop plants (He et al., 2016; Bi et al., 2016, 2017; Landi et al., 2017).

### **7.3 APPROACHES TO MANIPULATE GENE PATHWAYS USING TRANSCRIPTION FACTORS FOR ENHANCED ABIOTIC STRESS TOLERANCE**

Generation of transgenic plants represents a complementary approach to plant breeding for developing stress-tolerant crop plants, particularly drought stress. Many studies have reported that genetically engineered plants exhibited high tolerance to drought and associated stresses (Denby et al., 2005; Vinocur & Altman, 2005; Valliyodan & Nguyen, 2006; Yang et al., 2010; Funganti-Pagliarini et al., 2017; Wani et al., 2017). However, models' plants like *Arabidopsis* were used in most of these published results and researchers

later included some important crop plants such as rice, maize, soybean, barley (Seo et al., 2012). Because of the multigenetic nature of the stress tolerance in plants, a comprehensive understanding of stress tolerance at the molecular level is essential (Peleg & Blumwald, 2011; Zwack & Rashotte, 2015). Mittler & Blumwald (2010) defined central dogma of abiotic research as, *“deciphering how plants sense and acclimatize to an abiotic stress and using this knowledge to develop crops with enhanced tolerance to abiotic stresses.”* It is important to note that genetically modified (GM) crops available for commercial exploitation are based on single gene traits such as insect resistance or herbicide resistance.

TFs hold master switches status, actively control, and regulate several cellular and molecular processes in plant (Century et al., 2008; Hussain et al., 2011a). Genetic engineering efforts using TFs provides a systematic approach for improvement of crop plant to various abiotic stresses because TFs act as central regulators of several downstream stress related genes (Bihani et al., 2011; Datta et al., 2012; Guo et al., 2016; Hu et al., 2016; Zhu et al., 2018; Agarwal et al., 2019; Yang et al., 2019). Therefore, comprehensive knowledge on mechanistic regulation of TFs is important for future research (Seki et al., 2002; Yamaguchi-Shinozaki & Shinozaki, 2006). Moreover, genetic engineering of transcriptional networks seems to be a more precisely predictable and practical strategy compared to single structural gene engineering because of the multigenic nature of stress tolerance. Therefore, vast research data conclusively declared genetic engineering of TFs as an excellent approach for handling complex traits such as stress tolerance in crop plants (Century et al., 2008; Bhatnagar-Mathur et al., 2014; Hong et al., 2017). Several studies have shown promising results in improving crop tolerance to different stresses under controlled and field conditions (Hsieh et al., 2013; Liang et al., 2016). Seki et al. (2002) using Arabidopsis plant and high throughput microarray tool studied expression profile of several thousand genes (over 7,000 genes) and found that genes showing upregulation under various stresses belong to important families of TFs such as AP2/ERF, WRKY, MYB, NAC, HD-Zip, and bZIP (Ying et al., 2012; Zhu et al., 2018). Several members of TFs families, such as NAC, AP2/ERF, bZIP, and WRKY have been studied in transgenic model and crop plants demonstrated their involvement in adaptive responses to different abiotic stresses (Singh et al., 2002; Rushton et al., 2010). In this context, huge progress has been achieved by overexpressing different TFs genes in plants and exposed to multiple stresses (Saad et al., 2013; Xiong et al., 2014; Fang et al., 2015; Huang et al., 2015; Casaretto et al., 2016; He et al., 2016; Rahman et al., 2016; Zhang

et al., 2016; Yoon et al., 2017; Chung et al., 2018; Gao et al., 2018; Shim et al., 2018; Wang et al., 2018; El-Esawi et al., 2019a, b; Esmacili et al., 2019; Tang et al., 2019; Waqas et al., 2019). Recently, few published articles comprehensively reviewed TFs engineering in crop plants for abiotic stress tolerance (Baillo et al., 2019; Khan et al., 2019; Kimotho et al., 2019; Waqas et al., 2019; Javed et al., 2020; Ahmed et al., 2020; Hrmova & Hussain, 2021; Manna et al., 2021).

### **7.3.1 TRANSGENIC PLANTS OVEREXPRESSING TRANSCRIPTION FACTORS**

Comprehensive genome-wide analysis of several plant species has yielded a plethora of TFs belonging to various families (Cominelli et al., 2010; Yadav et al., 2011; Seo et al., 2012; Gao et al., 2017). Consequently, functional analysis of these TFs via knockout/knockdown analysis and overexpression of TFs in both model and crop plants have reported their roles in plants. Overexpression of TFs activates the expression of several downstream genes which control the multigenic traits such as tolerance to abiotic stresses (Seki et al., 2007; Hussain et al., 2011a). DREB gene family is the best studies TFs using overexpression approach in different plants such as rice, barley tobacco, soybean, wheat, tomato, potato, peanut, oilseed rape for abiotic stress tolerance (Kasuga et al., 1999, 2004; Pellegrineschi et al., 2004; Oh et al., 2005, 2007; Bhatnagar-Mathur et al., 2004, 2006, 2009; Behnam et al., 2006; Ito et al., 2006; Chen et al., 2007, 2008; Zhao et al., 2007; Cong et al., 2008; Wang et al., 2008). Similarly, several other TFs such as, MYB, WRKY, NAC, bZIP, AREB, ERF, and CBF have been utilized for developing stress-tolerant crop plants (Tran et al., 2004; Villalobos et al., 2004; Fujita et al., 2005; Furihata et al., 2006; Zhang et al., 2005, 2007; Dai et al., 2007; Liao et al., 2008; Trujillo et al., 2008; Zhou et al., 2008; Qiu & Yu, 2009; Yanez et al., 2009; Abdeen et al., 2010; Morran et al., 2010).

As mentioned above, DREB and other TFs are playing significant role in the regulation of abiotic stress tolerance in plants. For example, it is reported that overexpression of SNAC1 in rice showed higher seed setting (22–34%) in field conditions compared to control plants under drought stress. Transgenic rice plants provided high yield because plants demonstrated good control on closing of stomata leading to less water loss and good photosynthetic performance under stress. Similarly, Overexpression of OsNAC6/SNAC2 and ONAC045 conferred broad abiotic stress tolerance

(cold, salinity, and drought) in transgenic rice (Nakashima et al., 2007; Hu et al., 2008; Zheng et al., 2009). Studies have shown that Overexpression of three DREB1s/CBFs (DREB1A/CBF3, DREB1B/CBF1, and DREB1C/CBF2) significantly could improve tolerance to freezing, drought, and high salinity in transgenic Arabidopsis (Gilmour et al., 2004; Novillo et al., 2004). Overexpression of MYB15 in Arabidopsis plants improved salt and drought tolerance of transgenic plants by high expression of various stress-related proteins and stomatal closure under water shortage (Ding et al., 2009). High cuticular wax accumulation and enhanced drought tolerance was observed in transgenic plants overexpressing WXP1 and SHINE1-3 TFs which show that these TFs are involved in induction of wax related genes (Zhang et al., 2005, 2007). Yang et al. (2012a) reported that transgenic plants overexpressing OsMYB2 TF exhibited enhanced tolerance to various stresses (Drought, Salt, freezing) by altering expression levels of many downstream stress-responsive genes. Overexpression of two transcription factors from soybean, GmMYB76 or GmMYB177 in Arabidopsis led to salt and freezing tolerance (Liao et al., 2008). Qiu & Yu (2009) reported the Overexpression of OsWRKY45 in Arabidopsis conferred drought tolerance mainly due to stomatal closure and induction of downstream stress-related genes under drought stress. Similarly, rice and Arabidopsis plants overexpressing OsWRKY11 and VvWRKY exhibited significantly high heat, drought, and osmotic stress tolerance (Wu et al., 2009). Similarly, Arabidopsis transgenic plants overexpressing two soybean GmWRKY21, GmWRKY54 genes showed improved tolerance to cold, drought, and salt stresses (Zhou et al., 2008). In another attempt, Xiang et al. (2008) studied the overexpression of OsbZIP23 in rice. Expression analysis of transgenic plants showed changes in the expression of over 1,000 genes and many of these have significant roles in drought and salinity tolerance (Xiang et al., 2008). As a matter of fact, the above discussion highlighted the significant roles of transcription factors in gene regulation under different stresses which make these genes ideal candidates for engineering multiple abiotic stress tolerance in crop plants (Hussain et al., 2011a).

### **7.3.2 STRATEGIES FOR MANIPULATING TRANSCRIPTION FACTOR ACTIVITY IN TRANSGENIC PLANTS**

Constitutive overexpression of key regulatory proteins like TFs might show some undesirable negative effects such as stunted growth, unwanted

phenotype, yield penalty on normal plant development and growth (Liu et al., 1998; Kasuga et al., 1999, 2004; Ito et al., 2006; Nakashima et al., 2006; Gao et al., 2011; Conant et al., 2014; Reis et al., 2014). For example, researchers have noticed growth retardation in transgenic plants overexpressing DREB1A/CBF TF (Ito et al., 2006). These undesirable and other pleiotropic effects of TF overexpression have been attributed to the sensitivity of certain cell types to the overexpression. On the other hand, studies have shown that overexpression of TFs regulates the expression of several downstream genes, may lead to different phenotypic effects resulting in undesirable effects. Several studies have reported pleiotropic effects under circumstances when constitutively overexpressed TF resulted in altered expression of downstream target genes/stress-related genes which led to an undesirable altered phenotype of transgenic plants. Similarly, stunted growth is the most common undesirable effect in transgenic plants overexpressing TFs (Kasuga et al., 2004; Ito et al., 2006; Wu et al., 2009). However, pleiotropic effects are not always present in transgenic plants overexpressing TFs. For example, transgenic rice plants overexpressing DREB1A/CBF3 and OsDREB1F showed no stunted growth or any visible phenotypic alterations (Oh et al., 2005; Wang et al., 2008). Similarly, Oh et al. (2005) reported no pleiotropic effects in transgenic rice overexpressing basic leucine zipper (bZIP) transcription factor (ABF3).

Similarly, no pleiotropic effects were also observed for the bZIP transcription factor ABF3 when ectopically expressed in rice (Oh et al., 2005). Liu et al. (1998) reported an interesting observation that overexpression of certain TFs such as DREB2 was unable to induce stress tolerance (drought) in transgenic plants. DREB2 protein contains repressor domain which prevent the activation of downstream stress related genes. Sakuma et al. (2006) addressed this problem and showed that deletion of this repressor domain (deletion of a region between residues 136 and 165) stores the active form of AtDREB2A. Furthermore, overexpression of the active form of AtDREB2A in transgenic plants resulted up-regulation of several drought inducible downstream genes, which led to improved drought stress tolerance of transgenic Arabidopsis. Yoshida et al. (2008) demonstrated that overexpression of modified DREB2A (Sakuma et al., 2006) rendered transgenic plants tolerant to drought and salt stress but also led to enhanced heat tolerance of transgenic plants. This indicates the existence of crosstalk between drought and heat and that DREB2A controls the expression of HsfA3 (heat stress TF) (Schramm et al., 2008). Similarly, pleiotropic problems can be easily overcome by making a smart choice of either stress-inducible promoter (RD29A)

or tissue/cell-specific promoter (root-specific RCc3 promoter) (Nakashima et al., 2007). Thus, it may be particularly important to restrict TF activity with the judicious choice of promoters to minimize undesirable deleterious effects in plant engineering experiment. For example, overexpression of ZmDREB2A under either constitutive or stress-inducible promoter led to enhanced drought tolerance in transgenic maize plants (Qin et al., 2007).

It is now easy to make judicious choice of promoters because a plethora of different types of natural and purpose-built synthetic promoters are available for deriving gene expression depending on the objectives of the experiments. For example, transgenic wheat and barley plants overexpressing wheat DREB2 and DREB3 under both constitutive and drought inducible promoter were generated (Morran et al., 2010). The objective of the experiment was to study promoter which may help to increase the plant survival under stress without deleterious effect on plant growth. Gene expression driven by stress-inducible promoter showed no undesirable effect on plant growth whereas, constitutive expression of the gene under drought stress exhibited multiple unwanted phenotypic effects such as delayed flowering which led to low yield, stunted growth, and smaller spikes (Morran et al., 2010). Reichmann et al. (2000) reported systematic analysis of Arabidopsis TFs and identified putative candidate genes with potential active role in stress tolerance in plants. Nelson et al. (2007) pointed out that research results from Arabidopsis can be directly applied to crop plant improvement. Similarly, many studies have utilized the use of transcriptional repressor/activator which have the potential to become important genomic tools (Hiratsu et al., 2003; Fujita et al., 2005). In conclusion, modified TF based tools can be a potential resource in transgenic research, attributing novel traits to the transgenic plants, especially under environmental stresses.

### **7.3.3 CIS-TRANS ENGINEERING LEADING TO ARTIFICIAL TRANSCRIPTION FACTOR FOR ABIOTIC STRESS TOLERANCE**

Knowledge-based modifications in DNA binding domains (DBDs) for engineering TFs to meet particular needs require the use of current advances in molecular biology and other allied sciences. Currently, TF engineering (Trans engineering) strategy depends on introducing specific modifications in the TF sequence to enhance its binding interaction with designated target sequence (Gitzinger et al., 2009; Lu et al., 2009). The classical reprogrammable synthetic TFs depends on the creation of



specific Zinc Finger (ZF) domains (Beerli & Barbas, 2002; Lindhout et al., 2006). Researchers have extensively used this approach to fine-tune gene expression in different systems including plants (Maeder et al., 2008; Foley et al., 2009; Townsend et al., 2009; Mitsuda et al., 2011; vanTol et al., 2017). However, several major problems have been identified in the ZF domains functions. For example, designing and construction of ZF domains is labor intensive and extremely time-consuming and lack the desired target specificity. Machens et al. (2017) pointed out these inherent problems with ZF domains and further questioned the precise functioning of these domains for any endogenous promoter. Thus, desirable efficiency for regulation of any specific transcription module is not fully achieved (Machens et al., 2017).

On the other hand, Transcription activator-like effectors (TALEs) is another promising alternative approach to ZF domains. Studies have demonstrated this approach comparative target-oriented and more precise in TF engineering (Boch, 2011; Bogdanove & Voytas, 2011). Bagdanove & Voytas (2011) showed that TALEs have a relatively higher degree of specificity compared to ZF domains. The reason for high specificity is that TALEs are specifically designed using 18 out of 34 amino acids repeats that specify contiguous DNA nucleotides (Bogdanove & Voytas, 2011). TALEs have demonstrated advantages over ZF domains. However, constructing a new target specific protein for designing specific DBDs in TALEs is cumbersome. This limits the engineering of efficient ATFs for generating quick knockouts/mutations. However, many attempts have introduced several new characters in TALE design to increase its efficiency (Zhang et al., 2014; Lowder et al., 2017; Schwartz et al., 2017). Another problem is that TALEs have been shown to be sensitive to cytosine methylation and unsuitable for targets with CpG sites (Bogdanove & Voytas, 2011). CRISPR-Cas9 (clustered regularly interspaced short palindromic repeat-associated protein 9) technology represents another landmark discovery in tailoring the precise regulation of gene expression. This technology has circumvented ZF and TALEs related problems using a catalytically inactive/dead CRISPR associated protein 9 (dCas9) domain to engineer ATFs (Cheng et al., 2013; Lowder et al., 2015; Wyvekens et al., 2015). It is experimentally proved that dCas9 protein provide effective control of gene expression in a variety of prokaryotes and eukaryotes (Jinek et al., 2012; Wiedenheft et al., 2012; Cho et al., 2013; Cong et al., 2013; DiCarlo et al., 2013; Friedland et al., 2013; Hwang et al., 2013; Jiang et al., 2013a, b; Mali et al., 2013) including plant systems (Feng et al., 2013, 2014; Li et al., 2013a; Miao et al., 2013;

Nekrasov et al., 2013; Shan et al., 2013). However, innovations in the application of dCas9-mediated multiplexing have supported researchers to introduce desired transcriptional manipulations at a relatively faster pace compared to other methods.

## **7.4 CONCLUSION AND OUTLOOK**

Plants being sessile are exposed to different abiotic stresses simultaneously under natural environments. Plant survival in such environments depends on coordinated activation of multiple genes, signaling, and stress response pathways which lead to either synergistic or antagonistic effect on each other. Current progress on exploring the role of various plant TFs highlighted the importance in conferring abiotic stress tolerance. However, detailed knowledge of signaling networks, regulatory pathways and comprehensive functional performance of TFs is extremely important. In addition, concerted efforts should be focused on the genome-wide identification and functional characterization of multifunctional TFs genes to raise multiple stress-resistant crop plants.

Genome-wide identification and analysis has yielded a plethora of TFs involved in environmental stress tolerance. However, owing to the complexity of regulating networks among different TFs at different levels including functional validity, crosstalk, and interactions during abiotic stress require further targeted research. Deep understanding of functional perspectives about specific TFs, especially their downstream stress-responsive target genes. This information can potentially provide a better opportunity for exploration of regulatory networks involved in abiotic stress tolerance in plants. Taking together, detailed studies will support identifying potential TF genes and designing strategies for developing stress-tolerant crops. On the other hand, the availability of high throughput tools has allowed researcher to generate modified versions of TFs with improved DNA-binding properties. Furthermore, synthetically tailored TFs could be independent of upstream regulatory circuits. Generation of new knowledge will also help us to choose suitable TFs for bioengineering plants with high abiotic stress tolerance.

## KEYWORDS

- **abiotic stresses**
- **CRISPR-Cas9**
- **genome editing**
- **plant engineering**
- **signaling networks**
- **transcription factors**

## REFERENCES

- Abdeen, A., Schnell, J., & Miki, B., (2010). Transcriptome analysis reveals absence of unintended effects in drought-tolerant transgenic plants overexpressing the transcription factor ABF3. *BMC Genomics*, 11, 69.
- Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K., & YamaguchiShinozaki, K., (2003). *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell*, 15, 63–78.
- Agarwal, P., Baranwal, V. K., & Khurana, P., (2019). Genome-wide analysis of bZIP transcription factors in wheat and functional characterization of a *TabZIP* under abiotic stress. *Sci. Rep.*, 9, 1–18.
- Ahmad, R., Kim, M. D., Back, K. H., Kim, H. S., Lee, H. S., Kwon, S. Y., Murata, N., et al., (2008). Stress-induced expression of choline oxidase in potato plant chloroplasts confers enhanced tolerance to oxidative, salt, and drought stresses. *Plant Cell Rep.*, 27, 687–698.
- Ahmed, R. F., Irfan, M., Shakir, H. A., Khan, M., & Chen, L., (2020). Engineering drought tolerance in plants by modification of transcription and signaling factors. *Biotechnol. Biotechnol. Equip.*, 34, 781–789.
- Alcazar, R., Planas, J., Saxena, T., Zarza, X., Bortolotti, C., Cuevas, J., Bitrian, M., et al., (2010). Putrescine accumulation confers drought tolerance in transgenic *Arabidopsis* plants over-expressing the homologous arginine decarboxylase 2 gene. *Plant Physiol Biochem.*, 48, 547–552.
- Alexander, R. D., Wendelboe-Nelson, C., & Morris, P. C., (2019). The barley transcription factor *HvMYB1* is a positive regulator of drought tolerance. *Plant Physiology and Biochemistry*, 142, 246–253.
- Almeida, A. M., Silva, A. B., Araujo, S. S., Cardoso, L. A., Santos, D. M. J., Torne, J. M., Silva, J. M., et al., (2007). Responses to water withdrawal of tobacco plants genetically engineered with the *AtTPS1* gene: A special reference to photosynthetic parameters. *Euphytica*, 154, 113–126.
- Alvarez-Gerding, X., Espinoza, C., Inostroza-Blancheteau, C., & Arce-Johnson, P., (2015). Molecular and physiological changes in response to salt stress in *Citrus macrophylla* W plants overexpressing *Arabidopsis* CBF3/DREB1A. *Plant Physiol. Biochem.*, 92, 71–78.

- Alzahrani, F. O., (2021). Metabolic engineering of osmoprotectants to elucidate the mechanism(s) of salt stress tolerance in crop plants. *Planta*, 253, 24.
- Aroca, R., Porcel, R., & Ruiz-Lozano, J. M., (2012). Regulation of root water uptake under abiotic stress conditions. *J. Exp. Bot.*, 63, 43–57.
- Asgari, L. H., Savaghebi, G., Hadian, J., Hatami, M., & Pezhmanmehr, M., (2017). Comparison of copper and zinc effects on growth, micro-and macronutrients status and essential oil constituents in pennyroyal (*Mentha pulegium* L.). *Braz. J. Bot.*, 40, 379–388.
- Ashraf, M., & Foolad, M. R., (2007). Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.*, 59, 206–216.
- Atkinson, N. J., & Urwin, P. E., (2012). The interaction of plant biotic and abiotic stresses: From genes to the field. *J. Exp. Bot.*, 63, 3523–3543.
- Babitha, K. C., Ramu, S. V., Nataraja, K. N., Sheshshayee, M. S., & Udayakumar, M., (2015). *EcbZIP60*, a basic leucine zipper transcription factor from *Eleusine coracana* L. improves abiotic stress tolerance in tobacco by activating unfolded protein response pathway. *Mol. Breed.*, 35, 181.
- Badu-Apraku, B., & Yallou, C. G., (2009). Registration of striga resistant and drought tolerant tropical early maize populations TZE-W Pop DT STR C4 and TZE-Y Pop DT STR C4. *J. Plant Regist.*, 3, 86–90.
- Baillo, E. H., Kimotho, R. N., Zhang, Z., & Xu, P., (2019). Transcription factors associated with abiotic and biotic stress tolerance and their potential for crop improvement. *Genes*, 10, 771.
- Baldoni, E., Genga, A., & Cominelli, E., (2015). Plant MYB transcription factors: Their role in drought response mechanisms. *Int. J. Mol. Sci.*, 16, 15811–15851.
- Bartels, D., & Hussain, S. S., (2008). Current status and implications of engineering drought tolerance in plants using transgenic approaches. *CAB Rev. Persp. Agr. Vet. Sci. Nutri Natu. Sci.*, 3, 20.
- Bartels, D., & Sunkar, R., (2005). Drought and salt tolerance in plants. *CRC Crit. Rev. Plant Sci.*, 24, 23–58.
- Bauer, H., Ache, P., Lautner, S., Fromm, J., Hartung, W., Al-Rasheid, K. A., Sonnewald, S., et al., (2013). The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. *Curr. Biol.*, 23, 53–57.
- Baxter, A., Mittler, R., & Suzuki, N., (2014). ROS as key players in plant stress signaling. *J. Exp. Bot.*, 65, 1229–1240.
- Beerli, R. R., & Barbas, C. F. III., (2002). Engineering polydactyl zinc finger transcription factors. *Nat. Biotechnol.*, 20, 135.
- Behelgardy, M. F., Motamed, N., & Jazii, F. R., (2012). Expression of the P5CS gene in transgenic versus non-transgenic olive (*Olea europaea*) under salinity stress. *World Appl. Sci. J.*, 18, 580–583.
- Behnam, B., Kikuchi, A., Celebi-Toprak, F., Yamanaka, S., Kasuga, M., Yamaguchi-Shinozaki, K., & Watanabe, K. N., (2006). The *Arabidopsis DREB1A* gene driven by the stress-inducible *rd29A* promoter increases salt-stress tolerance in proportion to its copy number in tetrasomic tetraploid potato (*Solanum tuberosum*). *Plant Biotech.*, 23, 169–177.
- Bhargava, S., & Sawant, K., (2013). Drought stress adaptation: Metabolic adjustment and regulation of gene expression. *Plant Breed.*, 132, 21–32.
- Bhatnagar-Mathur, P., Devi, M. J., Reddy, D. S., Vadez, V., YamaguchiShinozaki, K., & Sharma, K. K., (2006). Overexpression of *Arabidopsis thaliana DREB1A* in transgenic peanut (*Arachis hypogaea* L.) for improving tolerance to drought stress (poster presentation).

- In: Arthur, M. S., (ed.), *'From Functional Genomics of Model Organisms to Crop Plants for Global Health*. Washington, DC: National Academy of Science.
- Bhatnagar-Mathur, P., Devi, M. J., Serraj, R., Yamaguchi-Shinozaki, K., Vadez, V., & Sharma, K. K., (2004). Evaluation of transgenic groundnut lines under water limited conditions. *Int. Arachis Newslett.*, 24, 33–34.
- Bhatnagar-Mathur, P., Devi, M. J., Vadez, V., & Sharma, K. K., (2009). Differential antioxidative responses in transgenic peanut bear no relationship to their superior transpiration efficiency under drought stress. *J. Plant Physiol.*, 166, 1207–1217.
- Bhatnagar-Mathur, P., Rao, J. S., Vadez, V., Dumbala, S. R., Rathore, A., Yamaguchi-Shinozaki, K., & Sharma, K. K., (2014). Transgenic peanut overexpressing the *DREB1A* transcription factor has higher yields under drought stress. *Mol. Breed.*, 33, 327–340.
- Bi, H., Luang, S., Li, Y., Bazanova, N., Borisjuk, N., Hrmova, M., & Lopato, S., (2017). Wheat drought-responsive WXPL transcription factors regulate cuticle biosynthesis genes. *Plant Mol. Biol.*, 94, 15–32.
- Bi, H., Luang, S., Li, Y., Bazanova, N., Morran, S., Song, Z., Perera, M. A., et al, (2016). Identification and characterization of wheat drought-responsive MYB transcription factors involved in the regulation of cuticle biosynthesis. *J. Exp. Bot.*, 67, 5363–5380.
- Bihani, P., Char, B., & Bhargava, S., (2011). Transgenic expression of sorghum *DREB2* in rice improves tolerance and yield under water limitation. *J. Agric. Sci.*, 149, 95–101.
- Boch, J., (2011). TALEs of genome targeting. *Nat. Biotechnol.*, 29, 135.
- Bogdanove, A. J., & Voytas, D. F., (2011). TAL effectors: Customizable proteins for DNA targeting. *Science*, 333, 1843–1846.
- Bohnert, H. J., & Jensen, R. G., (1996). Strategies for engineering water stress tolerance in plants. *Trends Biotechnol.*, 14, 89–97.
- Bortesi, L., & Fischer, R., (2015). The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol. Adv.*, 33, 41–52.
- Boston, R. S., Viitanen, P. V., & Vierling, E., (1996). Molecular chaperones and protein folding in plants. *Plant Mol. Biol.*, 32, 191–222.
- Boudsocq, M., & Sheen, J., (2013). CDPKs in immune and stress signaling. *Trends Plant Sci.*, 18, 30–40.
- Butt, H. I., Yang, Z., Chen, E., Zhao, G., Gong, Q., Yang, Z., Zhang, X., & Li, F., (2017a). Functional characterization of cotton *GaMYB62L*, a novel R2R3 TF in transgenic *Arabidopsis*. *PLoS One*, 12, e0170578.
- Butt, H. I., Yang, Z., Gong, Q., Chen, E., Wang, X., Zhao, G., Ge, X., et al., (2017b). *GaMYB85*, an R2R3 MYB gene, in transgenic *Arabidopsis* plays an important role in drought tolerance. *BMC Plant Biol.*, 17, 142.
- Cabello, J. V., Lodeyro, A. F., & Zurbriggen, M. D., (2014). Novel perspectives for the engineering of abiotic stress tolerance in plants. *Curr. Opin. Biotechnol.*, 26, 62–70.
- Cai, H., Tian, S., Dong, H., & Guo, C., (2015). Pleiotropic effects of *TaMYB3R1* on plant development and response to osmotic stress in transgenic *Arabidopsis*. *Gene*, 558, 227–234.
- Cai, R., Dai, W., Zhang, C., Wang, Y., Wu, M., Zhao, Y., Ma, Q., et al., (2017). The maize WRKY transcription factor *ZmWRKY17* negatively regulates salt stress tolerance in transgenic *Arabidopsis* plants. *Planta*, 246, 1215–1231.
- Cai, W., Yang, Y., Wang, W., Guo, G., Liu, W., & Bi, C., (2018). Overexpression of a wheat (*Triticum aestivum* L.) bZIP transcription factor gene, *TabZIP6*, decreased the freezing tolerance of transgenic *Arabidopsis* seedlings by down-regulating the expression of CBFs. *Plant Physiol. Biochem.*, 124, 100–111.

- Calvo-Polanco, M., Señorans, J., & Zwiazek, J. J., (2012). Role of adventitious roots in water relations of tamarack (*Larix laricina*) seedlings exposed to flooding. *BMC Plant Biol.*, 12, 1.
- Campos, J. F., Cara, B., Perez-Martin, F., Pineda, B., Egea, I., Flores, F. B., Fernandez-Garcia, N., Cape, J., Moreno, V., Angosto, T., et al., (2016). The tomato mutant *ars1* (altered response to salt stress 1) identifies an R1-type MYB transcription factor involved in stomatal closure under salt acclimation. *Plant Biotechnol. J.*, 14, 1345–1356.
- Cao, L., Yu, Y., Ding, X., Zhu, D., Yang, F., Liu, B., Sun, X., et al., (2017). The *Glycine soja* NAC transcription factor *GsNAC019* mediates the regulation of plant alkaline tolerance and ABA sensitivity. *Plant Mol. Biol.*, 95, 253–268.
- Capell, T., Bassie, L., & Christou, P., (2004). Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. *Proc. Natl. Acad. Sci. USA*, 101, 9909–9914.
- Casaretto, J. A., El-kereamy, A., Zeng, B., Stiegelmeier, S. M., Chen, X., Bi, Y. M., & Rothstein, S. J., (2016). Expression of *OsMYB55* in maize activates stress-responsive genes and enhances heat and drought tolerance. *BMC Genom.*, 17, 312.
- Century, K., Reuber, T. L., & Ratcliffe, O. J., (2008). Regulating the regulators: The future prospects for transcription-factor-based agricultural biotechnology products. *Plant Physiol.*, 147, 20–29.
- Chang, Y., Nguyen, B. H., Xie, Y., Xiao, B., Tang, N., Zhu, W., Mou, T., & Xiong, L., (2017). Co-overexpression of the constitutively active form of *OsbZIP46* and ABA-activated protein kinase SAPK6 improves drought and temperature stress resistance in rice. *Front. Plant Sci.*, 8, 1102.
- Chanwala, J., Satpati, S., Dixit, A., Parida, A., Giri, M. K., & Dey, N., (2020). Genome-wide identification and expression analysis of WRKY transcription factors in pearl millet (*Pennisetum glaucum*) under dehydration and salinity stress. *BMC Genom.*, 21, 1–16.
- Chaves, M. M., & Oliveira, M. M., (2004). Mechanisms underlying plant resistance to water deficits: Prospects for water-saving agriculture. *J. Exp. Bot.*, 55, 2365–2384.
- Chen, D., Chai, S., McIntyre, C. L., & Xue, G. P., (2018). Overexpression of a predominantly root-expressed NAC transcription factor in wheat roots enhances root length, biomass and drought tolerance. *Plant Cell Rep.*, 37, 225–237.
- Chen, H. C., Cheng, W. H., Hong, C. Y., Chang, Y. S., & Chang, M. C., (2018). The transcription factor *OsbHLH035* mediates seed germination and enables seedling recovery from salt stress through ABA-dependent and ABA-independent pathways, respectively. *Rice*, 11, 50.
- Chen, H. C., Hsieh-Feng, V., Liao, P. C., Cheng, W. H., Liu, L. Y., Yang, Y. W., Lai, M. H., & Chang, M. C., (2017). The function of *OsbHLH068* is partially redundant with its homolog, *AtbHLH112*, in the regulation of the salt stress response but has opposite functions to control flowering in *Arabidopsis*. *Plant Mol. Biol.*, 94, 531–548.
- Chen, J. B., Wang, S. M., Jing, R. L., & Mao, X. G., (2009). Cloning the *PvP5CS* gene from common bean (*Phaseolus vulgaris*) and its expression patterns under abiotic stresses. *J. Plant Physiol.*, 166, 12–19.
- Chen, J. Q., Meng, X. P., Zhang, Y., Xia, M., & Wang, X. P., (2008). Over-expression of *OsDREB* genes lead to enhanced drought tolerance in rice. *Biotechnol. Lett.*, 30, 2191–2198.
- Chen, L., Song, Y., Li, S., Zhang, L., Zou, C., & Yu, D., (2012). The role of WRKY transcription factors in plant abiotic stresses. *Biochim. Biophys. Acta*, 1819, 120–128.

- Chen, M., Wang, Q. Y., Cheng, X. G., Xu, Z. S., Li, L. C., Ye, X. G., Xia, L. Q., & Ma, Y. Z., (2007). *GmDREB2*, a soybean DRE-binding transcription factor, conferred drought and high-salt tolerance in transgenic plants. *Biochem. Biophys. Res. Commun.*, 353, 299–305.
- Chen, Z., Fang, X., Yuan, X., Zhang, Y., Li, H., Zhou, Y., & Cui, X., (2021). Overexpression of transcription factor *GmTGA15* enhances drought tolerance in transgenic soybean hairy roots and *Arabidopsis* plants. *Agronomy*, 11, 170.
- Cheng, L., Li, X., Huang, X., Ma, T., Liang, Y., Ma, X., Peng, X., et al., (2013). Overexpression of sheep grass R1-MYB transcription factor *LcMYB1* confers salt tolerance in transgenic *Arabidopsis*. *Plant Physiol. Biochem.*, 70, 252–260.
- Cho, S. W., Kim, S., Kim, J. M., & Kim, J. S., (2013). Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nat. Biotechnol.*, 31, 230–232.
- Chung, P. J., Jung, H., Choi, Y. D., & Kim, J. K., (2018). Genome-wide analyses of direct target genes of four rice NAC-domain transcription factors involved in drought tolerance. *BMC Genom.*, 19, 40.
- Claussen, W., (2005). Proline as a measure of stress in tomato plants. *Plant Sci.*, 168, 241–248.
- Cominelli, E., Galbiati, M., & Tonelli, C., (2010). Transcription factors controlling stomatal movements and drought tolerance. *Transcription*, 1, 41–45.
- Conant, G. C., Birchler, J. A., & Pires, J. C., (2014). Dosage, duplication, and diploidization: Clarifying the interplay of multiple models for duplicate gene evolution over time. *Curr. Opin. Plant Biol.*, 19, 91–98.
- Cong, L., Ran, F. A., Cox, D., Lin, S. L., Barretto, R., Habib, N., Hsu, P. D., et al., (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science*, 339, 819–823.
- Cong, L., Zheng, H. C., Zhang, Y. X., & Chai, T. Y., (2008). *Arabidopsis DREB1A* confers high salinity tolerance and regulates the expression of GA dioxygenases in tobacco. *Plant Sci.*, 174, 56–164.
- Corrales, A., Carrillo, L., Lasierra, P., Nebauer, S. G., Dominguez-Figueroa, J., Renau-Morata, B., Pollmann, S., et al., (2017). Multifaceted role of cycling DOF factor 3 (CDF3) in the regulation of flowering time and abiotic stress responses in *Arabidopsis*. *Plant Cell Environ.*, 40, 748–764.
- Cortina, C., & Culianez-Macia, F., (2005). Tomato abiotic stress enhanced tolerance by trehalose synthesis. *Plant Sci.*, 169, 75–82.
- Cui, M., Yoo, K., Hyoung, S., Nguyen, H., Kim, Y., Kim, H., Ok, S., et al., (2013). An *Arabidopsis* R2R3-MYB transcription factor, *AtMYB20*, negatively regulates type 2C serine/threonine protein phosphatases to enhance salt tolerance. *FEBS Lett.*, 587, 1773–1778.
- Cui, Y., Chen, C. L., Cui, M., Zhou, W. J., Wu, H. L., & Ling, H. Q., (2018). Four *IVabHLH* transcription factors are novel interactors of FIT and mediate JA inhibition of iron uptake in *Arabidopsis*. *Mol. Plant.*, 11, 1166–1183.
- Dai, X., Wang, Y., & Zhang, W. H., (2016). OsWRKY74, a WRKY transcription factor, modulates tolerance to phosphate starvation in rice. *J. Exp. Bot.*, 67, 947–960.
- Dai, X., Xu, Y., Ma, Q., Xu, W., Wang, T., Xue, Y., & Chong, K., (2007). Overexpression of an R1R2R3 MYB Gene, *OsMYB3R-2*, increases tolerance to freezing, drought, and salt stress in transgenic *Arabidopsis*. *Plant Physiol.*, 143, 1739–1751.
- Dang, F. F., Wang, Y. N., Yu, L., Eulgem, T., Lai, Y., Liu, Z. Q., Wang, X., et al., (2013). *CaWRKY40*, a WRKY protein of pepper, plays an important role in the regulation of tolerance to heat stress and resistance to *Ralstonia solanacearum* infection. *Plant Cell Environ.*, 36, 757–774.

- Danquah, A., De Zelicourt, A., Colcombet, J., & Hirt, H., (2014). The role of ABA and MAPK signaling pathways in plant abiotic stress responses. *Biotechnol. Adv.*, 32, 40–52.
- Datta, K., Baisakh, N., Ganguly, M., Krishnan, S., Yamaguchi-Shinozaki, K., & Datta, S. K., (2012). Overexpression of *Arabidopsis* and rice stress genes' inducible transcription factor confers drought and salinity tolerance to rice. *Plant Biotechnol. J.*, 10, 579–586.
- De Clercq, I., Vermeirssen, V., Van, A. O., Vandepoele, K., Murcha, M. W., Law, S. R., Inzé, A., et al., (2013). The membrane-bound NAC transcription factor *ANAC013* functions in mitochondrial retrograde regulation of the oxidative stress response in *Arabidopsis*. *Plant Cell*, 25, 3472–3490.
- Deikman, J., Petracek, M., & Heard, J. E., (2011). Drought tolerance through biotechnology: Improving translation from the laboratory to farmer's fields. *Curr. Opin. Biotechnol.*, 23, 243–250.
- Denby, K., & Gehring, C., (2005). Engineering drought and salinity tolerance in plants: Lessons from genome-wide expression profiling in *Arabidopsis*. *Trends Biotechnol.*, 23, 9–14.
- Diao, W. P., Snyder, J. C., Wang, S. B., Liu, J. B., Pan, B. G., Guo, G. J., & Wei, G., (2016). Genome-wide identification and expression analysis of WRKY gene family in *Capsicum annum* L. *Front. Plant Sci.*, 7(10), 3389.
- DiCarlo, J. E., Norville, J. E., Mali, P., Rios, X., Aach, J., & Church, G. M., (2013). Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic Acids Res.*, 41, 4336–4343.
- Ding, Z., Li, S., An, X., Liu, X., Qin, H., & Wang, D., (2009). Transgenic expression of MYB15 confers enhanced sensitivity to abscisic acid and improved drought tolerance in *Arabidopsis thaliana*. *J. Genet. Genomics*, 36, 17–29.
- Dong, J., Cao, L., Zhang, X., Zhang, W., Yang, T., Zhang, J., & Che, D., (2021). An R2R3-MYB transcription factor *RmMYB108* responds to chilling stress of *Rosa multiflora* and conferred cold tolerance of *Arabidopsis*. *Front Plant Sci.*, 12, 696919.
- Dong, Q., Zheng, W., Duan, D., Huang, D., Wang, Q., Liu, C., Li, C., et al., (2020). *MdWRKY30*, a group IIa WRKY gene from apple, confers tolerance to salinity and osmotic stresses in transgenic apple callus and *Arabidopsis* seedlings. *Plant Sci.*, 299, 110611.
- Dong, Y., Wang, C., Han, X., Tang, S., Liu, S., Xia, X., & Yin, W., (2014). A novel bHLH transcription factor *PebHLH35* from *Populus euphratica* confers drought tolerance through regulating stomatal development, photosynthesis and growth in *Arabidopsis*. *Biochem. Biophys. Res. Commun.*, 450, 453–458.
- Du, C., Zhao, P., Zhang, H., Li, N., Zheng, L., & Wang, Y., (2017). The *Reaumuria trigyna* transcription factor *RtWRKY1* confers tolerance to salt stress in transgenic *Arabidopsis*. *J. Plant Physiol.* 215, 48–58.
- Du, X., He, F., Zhu, B., et al., (2020). NAC transcription factors from *Aegilops markgrafi* reduce cadmium concentration in transgenic wheat. *Plant Soil*, 449, 39–50.
- Duan, M., Zhang, R., Zhu, F., Zhang, Z., Gou, L., Wen, J., Dong, J., & Wang, T., (2017). A lipid-anchored NAC transcription factor is translocated into the nucleus and activates glyoxalase I expression during drought stress. *Plant Cell*, 29, 1748–1772.
- Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., & Lepiniec, L., (2010). MYB transcription factors in *Arabidopsis*. *Trends Plant Sci.*, 15, 573–581.
- Ebrahimian-Motlagh, S., Ribone, P. A., Thirumalaikumar, V. P., Allu, A. D., Chan, R. L., Mueller-Roeber, B., & Balazadeh, S., (2017). *Jungbrunnen1* confers drought tolerance downstream of the HD-Zip I transcription factor *AtHB13*. *Front. Plant Sci.*, 8, 2118.



- Ehong, Y., Ezhang, H., Ehuang, L., Eli, D., & Esong, F., (2016). Overexpression of a stress-responsive NAC transcription factor gene *ONAC022* improves drought and salt tolerance in rice. *Front. Plant Sci.*, 7, 4.
- El-Esawi, M. A., Al-Ghamdi, A. A., Ali, H. M., & Ahmad, M., (2019b). Overexpression of *AtWRKY30* transcription factor enhances heat and drought stress tolerance in wheat (*Triticum aestivum* L.). *Genes*, 10, 163.
- El-Esawi, M., & Alayafi, A., (2019a). Overexpression of *StDREB2* transcription factor enhances drought stress tolerance in cotton (*Gossypium barbadense* L.). *Genes*, 10, 142.
- Esmaili, N., Yang, X., Cai, Y., Sun, L., Zhu, X., Shen, G., Payton, P., et al., (2019). Co-overexpression of *AVP1* and *OsSIZ1* in *Arabidopsis* substantially enhances plant tolerance to drought, salt, and heat stresses. *Sci. Rep.*, 9, 1–15.
- Fang, L., Su, L., Sun, X., Li, X., Sun, M., Karungo, S. K., Fang, S., et al., (2016). Expression of *Vitis amurensis* *NAC26* in *Arabidopsis* enhances drought tolerance by modulating jasmonic acid synthesis. *J. Exp. Bot.*, 67, 2829–2845.
- Fang, Q., Wang, X., Wang, H., Tang, X., Liu, C., Yin, H., Ye, S., et al., (2020). The poplar R2R3 MYB transcription factor *PtrMYB94* coordinates with abscisic acid signaling to improve drought tolerance in plants. *Tree Physiol.*, 40, 46–59.
- Fang, Y., Liao, K., Du, H., Xu, Y., Song, H., Li, X., & Xiong, L., (2015). A stress-responsive NAC transcription factor *SNAC3* confers heat and drought tolerance through modulation of reactive oxygen species in rice. *J. Exp. Bot.*, 66, 6803–6817.
- Feng, Z. Y., Zhang, B. T., Ding, W. N., Liu, X. D., Yang, D. L., Wei, P. L., Cao, F. Q., et al., (2013). Efficient genome editing in plants using a CRISPR/Cas system. *Cell Res.*, 23, 1229–1232.
- Feng, Z., Mao, Y., Xu, N., Zhang, B., Wei, P., Yang, D. L., Wang, Z., et al., (2014). Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas induced gene modifications in *Arabidopsis*. *Proc. Natl Acad. Sci. USA*, 111, 4632–4637.
- Fleury, D., Jefferies, S., Kuchel, H., & Langridge, P., (2010). Genetic and genomic tools to improve drought tolerance in wheat. *J. Exp. Bot.*, 61, 3211–3222.
- Foley, J. E., Yeh, J. R. J., Maeder, M. L., Reyon, D., Sander, J. D., Peterson, R. T., & Joung, J. K., (2009). Rapid mutation of endogenous zebrafish genes using zinc finger nucleases made by oligomerized pool engineering. *PLoS One*, 4, e4348.
- Friedland, A. E., Tzur, Y. B., Esvelt, K. M., Colaiacovo, M. P., Church, G. M., & Calarco, J. A., (2013). Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. *Nat. Methods*, 10, 741–743.
- Fritsche-Neto, R., & Borem, A., (2012). In: Fritsche-Neto, R., & Borem, A., (eds.), *Plant Breeding for Abiotic Stress Tolerance*. Springer Berlin Heidelberg, Berlin, Heidelberg, Heidelberg.
- Fuganti-Pagliarini, R., Ferreira, L. C., Rodrigues, F. A., Molinari, H. B. C., Marin, S. R. R., Molinari, M. D. C., Marcolino-Gomes, J., et al., (2017). Characterization of soybean genetically modified for drought tolerance in field conditions. *Front Plant Sci.*, 8, 1–15.
- Fujita, T., Maggio, A., Garcia-Rios, M., Bressan, R. A., & Csonka, L. N., (1998). Comparative analysis of the regulation of expression and structures of two evolutionarily divergent genes from D1-pyrroline-5-carboxylate synthetase from tomato. *Plant Physiol.*, 118, 661–674.
- Fujita, Y., Fujita, M., Satoh, R., Maruyama, K., Parvez, M., Seki, M., Hiratsu, K., et al., (2005). AREB1 is a transcriptional activator of novel ABRE dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. *Plant Cell*, 17, 3470–3488.

- Fujita, Y., Fujita, M., Shinozaki, K., & Yamaguchi-Shinozaki, K., (2011). ABA-mediated transcriptional regulation in response to osmotic stress in plants. *J. Plant Res.*, 124, 509–525.
- Fujita, Y., Nakashima, K., Yoshida, T., Fujita, M., Shinozaki, K., & Yamaguchi-Shinozaki, K., (2014). Role of abscisic acid signaling in drought tolerance and pre-harvest sprouting under climate change. In: Tuteja, N., & Gill, S. S., (eds.), *Climate Change and Plant Abiotic Stress Tolerance* (p. 521553). Wiley-VCH Verlag GmbH and Co. Weinheim Germany.
- Fujita, Y., Yoshida, T., & Yamaguchi-Shinozaki, K., (2013). Pivotal role of the AREB/ABF–SnRK2 pathway in ABRE mediated transcription in response to osmotic stress in plants. *Physiol. Plant.*, 147, 15–27.
- Furihata, T., Maruyama, K., Fujita, Y., Umezawa, T., Yoshida, R., Shinozaki, K., & Yamaguchi, S. K., (2006). Absciscic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Proc. Natl. Acad. Sci. USA*, 103, 1988–1993.
- Gao, F., Zhou, J., Deng, R. Y., Zhao, H. X., Li, C. L., Chen, H., Suzuki, T., Park, S. U., & Wu, Q., (2017). Overexpression of a Tartary buckwheat R2R3-MYB transcription factor gene, *FtMYB9*, enhances tolerance to drought and salt stresses in transgenic *Arabidopsis*. *J. Plant Physiol.*, 214, 81–90.
- Gao, H., Wang, Y., Xu, P., & Zhang, Z., (2018). Overexpression of a WRKY transcription factor *TaWRKY2* enhances drought stress tolerance in transgenic wheat. *Front. Plant Sci.*, 9, 997.
- Gao, S. Q., Chen, M., Xu, Z. S., Zhao, C. P., Li, L., Xu, H. J., Tang, Y. M., et al., (2011). The soybean *GmbZIP1* transcription factor enhances multiple abiotic stress tolerances in transgenic plants. *Plant Mol. Biol.*, 75, 537–553.
- Garg, A. K., Kim, J. K., Owens, T. G., Ranwala, A. P., Choi, Y. D., Kochian, L. V., & Wu, R. J., (2002). Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl. Acad. Sci. USA*, 99, 15898–15903.
- Gilmour, S. J., Fowler, S. G., & Thomashow, M. F., (2004). *Arabidopsis* transcriptional activators CBF1, CBF2, and CBF3 have matching functional activities. *Plant Mol. Biol.*, 54, 767–781.
- Gitzinger, M., Parsons, J., Reski, R., & Fussenegger, M., (2009). Functional cross-kingdom conservation of mammalian and moss (*Physcomitrella patens*) transcription, translation and secretion machineries. *Plant Biotechnol. J.*, 7, 73–86.
- Goddijn, O. J. M., & Van, D. K., (1999). Trehalose metabolism in plants. *Trends Plant Sci.*, 4, 315–319.
- Goel, D., Singh, A. K., Yadav, V., Babbar, S. B., Murata, N., & Bansal, K. C., (2011). Transformation of tomato with a bacterial *codA* gene enhances tolerance to salt and water stresses. *J. Plant Physiol.*, 168, 1286–1294.
- Gong, Q., Li, S., Zheng, Y., Duan, H., Xiao, F., Zhuang, Y., He, J., et al., (2020). SUMOylation of MYB30 enhances salt tolerance by elevating alternative respiration via transcriptionally upregulating AOX1a in *Arabidopsis*. *Plant J.*, 102, 1157–1171.
- Gong, X., Zhang, J., Hu, J., Wang, W., Wu, H., Zhang, Q., & Liu, J. H., (2015). *FcWRKY70*, a WRKY protein of *Fortunella crassifolia*, functions in drought tolerance and modulates putrescine synthesis by regulating arginine decarboxylase gene. *Plant Cell Environ.*, 38, 2248–2262.
- Grelet, J., Benamar, A., Teyssier, E., Avelange-Macherel, M. H., Grunwald, D., & Macherel, D., (2005). Identification in pea seed mitochondria of a late-embryogenesis abundant protein able to protect enzymes from drying. *Plant Physiol.*, 137, 157–167.

- Guan, C., Huang, Y. H., Cen, H. F., Cui, X., Tian, D. Y., & Zhang, Y. W., (2019). Overexpression of the *Lolium perenne* L. delta-1-pyrroline 5-carboxylate synthase (LpP5CS) gene results in morphological alterations and salinity tolerance in switchgrass (*Panicum virgatum* L.). *PLoS One*, 14, e0219669.
- Guan, C., Huang, Y. H., Cui, X., Liu, S. J., Zhou, Y. Z., & Zhang, Y. W., (2018). Overexpression of gene encoding the key enzyme involved in prolinebiosynthesis (PuP5CS) to improve salt tolerance in switchgrass (*Panicum virgatum* L.). *Plant Cell Rep.*, 37, 1187–1199.
- Gubiš, J., Vaňková, R., Červená, V., Dragúňová, M. M., Hudcovicová, M., Lichtnerová, H. T., Dokupil, T., & Jureková, Z., (2007). Transformed tobacco plants with increased tolerance to drought. *South Afr. J. Bot.*, 73, 505–511.
- Guerzoni, J. T. S., Belintani, N. G., Moreira, R. M. P., Hoshino, A. A., Domingues, D. S., Filho, J. C. B., & Vieira, L. G. E., (2014). Stress-induced  $\Delta$ 1-pyrroline-5-carboxylate synthetase (P5CS) gene confers tolerance to salt stress in transgenic sugarcane. *Acta Physiol. Plant.*, 36, 2309–2319.
- Guo, B., Wei, Y., Xu, R., Lin, S., Luan, H., Lv, C., Zhang, X., et al., (2016). Genome-wide analysis of APETALA2/ethylene-responsive factor (AP2/ERF) gene family in barley (*Hordeum vulgare* L.). *PLoS One*, 11, e0161322.
- Guo, C., Yao, L., You, C., Wang, S., Cui, J., Ge, X., & Ma, H., (2016). *MIDI* plays an important role in response to drought stress during reproductive development. *Plant J.*, 88, 280–293.
- Guo, L., Yang, H., Zhang, X., & Yang, S., (2013). Lipid transfer protein 3 as a target of *MYB96* mediates freezing and drought stress in *Arabidopsis*. *J. Exp. Bot.*, 64, 1755–1767.
- Guo, W., Zhang, J., Zhang, N., Xin, M., Peng, H., Hu, Z., Ni, Z., & Du, J., (2015). The wheat NAC transcription factor *TaNAC2L* is regulated at the transcriptional and post-translational levels and promotes heat stress tolerance in transgenic *Arabidopsis*. *PLoS One*, 10, e0135667.
- Guttikonda, S. K., Valliyodan, B., Neelakandan, A. K., Tran, L. S., Kumar, R., Quach, T. N., Voothuluru, P., et al., (2014). Overexpression of *AtDREB1D* transcription factor improves drought tolerance in soybean. *Mol. Biol. Rep.*, 41, 7995–8008.
- Haley, S. D., Johnson, J. J., Peairs, F. B., Quick, J. S., Stromberger, J. A., Clayshulte, S. R., Butler, J. D., et al., (2007). Registration of ‘ripper’ wheat. *J. Plant Regist.*, 1, 1–6.
- Han, D., Du, M., Zhou, Z., Wang, S., Li, T., Han, J., Xu, T., & Yang, G., (2020). Overexpression of a *Malus baccata* NAC transcription factor gene *MbNAC25* increases cold and salinity tolerance in *Arabidopsis*. *Int. J. Mol. Sci.*, 21, 1198.
- Hartl, F. U., (1996). Molecular chaperones in cellular protein folding. *Nature*, 381, 571–579.
- Hartmann, L., Pedrotti, L., Weiste, C., Fekete, A., Schierstaed, J., Göttler, J., Kempa, S., et al., (2015). Crosstalk between two bzip signaling pathways orchestrates salt induced metabolic reprogramming in *Arabidopsis* roots. *Plant Cell*, 27, 2244–2260.
- Hasegawa, P. M., Bressan, R., Zhu, J. K., & Bohnert, H. J., (2000). Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 51, 463–499.
- He, G. H., Xu, J. Y., Wang, Y. X., Liu, J. M., Li, P. S., Chen, M., Ma, Y. Z., & Xu, Z. S., (2016). Drought-responsive WRKY transcription factor genes *TaWRKY1* and *TaWRKY33* from wheat confer drought and/or heat resistance in *Arabidopsis*. *BMC Plant Biol.*, 16, 1–16.
- He, K., Zhao, X., Chi, X., Wang, Y., Jia, C., Zhang, H., Zhou, G., & Hu, R., (2019). A novel miscanthus NAC transcription factor *MINAC10* enhances drought and salinity tolerance in transgenic *Arabidopsis*. *J. Plant Physiol.*, 233, 84–93.

- He, L., Shi, X., Wang, Y., Guo, Y., Yang, K., & Wang, Y., (2016). *Arabidopsis* ANAC069 binds to C[A/G]CG[T/G] sequences to negatively regulate salt and osmotic stress tolerance. *Plant Mol. Biol.*, 93, 369–387.
- Hendrick, J. P., & Hartl, F. U., (1993). Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem.*, 62, 349–384.
- Hichri, I., Muhovski, Y., Zizkova, E., Dobrev, P. I., Gharbi, E., Franco-Zorrilla, J. M., Lopez-Vidriero, I., Solano, R., et al., (2017). The *Solanum lycopersicum* WRKY3 transcription factor SIWRKY3 is involved in salt stress tolerance in tomato. *Front. Plant Sci.*, 8, 1343.
- Hinz, M., Wilson, I. W., Yang, J., Buerstenbinder, K., Llewellyn, D., Dennis, E. S., Sauter, M., & Dolferus, R., (2010). *Arabidopsis* RAP2.2: An ethylene response transcription factor that is important for hypoxia survival. *Plant Physiol.*, 153, 757–772.
- Hiratsu, K., Matsui, K., Koyama, T., & Ohme-Takagi, M., (2003). Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in *Arabidopsis*. *Plant J.*, 34, 733–739.
- Ho, H. L., (2015). Functional roles of plant protein kinases in signal transduction pathways during abiotic and biotic stress. *J. Biodivers. Bioprospect. Dev.*, 2, 2.
- Hoang, X. L. T., Nguyen, N. C., Nguyen, Y. H., Watanabe, Y., Tran, L. P., & Thao, N. P., (2019). The soybean GmNAC019 transcription factor mediates drought tolerance in *Arabidopsis*. *Int. J. Mol. Sci.*, 21, 286.
- Hoang, X. L. T., Nhi, D. N. H., Thu, N. B. A., Thao, N. P., & Tran, L. S. P., (2017). Transcription factors and their roles in signal transduction in plants under abiotic stresses. *Curr. Genom.*, 18, 487–493.
- Hong, C., Cheng, D., Zhang, G., Zhu, D., Chen, Y., & Tan, M., (2017). The role of ZmWRKY4 in regulating maize antioxidant defense under cadmium stress. *Biochem. Biophys. Res. Commun.*, 482, 1504–1510.
- Hong, Y., Zhang, H., Huang, L., Li, D., & Song, F., (2016). Overexpression of a stress-responsive NAC transcription factor gene ONAC022 improves drought and salt tolerance in rice. *Front. Plant Sci.*, 7, 4.
- Hrmova, M., & Hussain, S. S., (2021). Plant transcription factors involved in drought and associated stresses. *International Journal of Molecular Sciences*, 22, 5662.
- Hrmova, M., & Lopato, S., (2014). Enhancing abiotic stress tolerance in plants by modulating properties of stress responsive transcription factors. In: Tuberosa, R., Granerm, A., & Frisonm, E., (eds.), *Genomics of Plant Genetic Resources. Part II: Crop Productivity, Food Security and Nutritional Quality* (Vol. 2, pp. 291–316). Springer: Dordrecht, The Netherlands.
- Hsieh, E. J., Cheng, M. C., & Lin, T. P., (2013). Functional characterization of an abiotic stress-inducible transcription factor AtERF53 in *Arabidopsis thaliana*. *Plant Mol. Biol.*, 82, 223–237.
- Hu, H., & Xiong, L., (2014). Genetic engineering and breeding of drought resistant crops. *Annu. Rev. Plant Biol.*, 65, 715–741.
- Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., & Xiong, L., (2006). Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc. Natl. Acad. Sci. USA.*, 103, 12987–12992.
- Hu, H., You, J., Fang, Y., Zhu, X., Qi, Z., & Xiong, L., (2008). Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. *Plant Mol. Biol.*, 67, 169–181.

- Hu, W., Yang, H., Yan, Y., Wei, Y., Tie, W., Ding, Z., & Zuo, J., (2016). Genome-wide characterization and analysis of bZIP transcription factor gene family related to abiotic stress in cassava. *Nature*, 7, 22783.
- Hu, X. J., Zhang, Z. B., Xu, P., Fu, Z. Y., Hu, S. B., & Song, W. Y., (2010). Multifunctional genes: The cross talk among the regulation networks of abiotic stress responses. *Biol. Plant.*, 54, 213–223.
- Huang, C., Zhou, J., Jie, Y., Xing, H., Zhong, Y., Yu, W., She, W., et al., (2016). A ramie bZIP transcription factor *BnbZIP2* is involved in drought, salt, and heavy metal stress response. *DNA Cell Biol.*, 35, 776–786.
- Huang, D., & Dai, W., (2015). Molecular characterization of the basic helix-loop-helix (bHLH) genes that are differentially expressed and induced by iron deficiency in *Populus*. *Plant Cell Rep.*, 34, 1211–1224.
- Huang, G. T., Ma, S. L., Bai, L. P., Zhang, L., Ma, H., Jia, P., Liu, J., et al., (2012). Signal transduction during cold, salt, and drought stresses in plants. *Mol. Biol. Rep.*, 39, 969–987.
- Huang, Q., Wang, Y., Li, B., Chang, J., Chen, M., Li, K., Yan, G., & He, G., (2015). *TaNAC29*, a NAC transcription factor from wheat, enhances salt and drought tolerance in transgenic *Arabidopsis*. *BMC Plant Biol.*, 15, 1–15.
- Huang, X., Li, K., Xu, X., Yao, Z., Jin, C., & Zhang, S., (2015). Genome-wide analysis of WRKY transcription factors in white pear (*Pyrus bretschneideri*) reveals evolution and patterns under drought stress. *BMC Genom.*, 16, 1–14.
- Huo, T., Wang, C. T., Yu, T. F., Wang, D. M., Li, M., Zhao, D., Li, X. T., et al., (2021). Overexpression of *ZmWRKY65* transcription factor from maize confers stress resistances in transgenic *Arabidopsis*. *Sci. Rep.*, 11, 4024.
- Hussain, S. S., Iqbal, M. T., Arif, M. A., & Amjad, M., (2011b). Beyond osmolytes and transcription factors: Drought tolerance in plants via protective proteins and aquaporins. *Biol. Plant.*, 55, 401–413.
- Hussain, S. S., Kayani, M. A., & Amjad, M., (2011a). Transcription factors as tools to engineer enhanced drought stress tolerance in plants. *Biotechnol. Prog.*, 27, 297–306.
- Hussain, S. S., Raza, H., Afzal, I., & Kayani, M. A., (2012). Transgenic plants for abiotic stress tolerance: Current status. *Arch. Agron. Soil Sci.*, 58, 693–721.
- Hwang, W. Y., Fu, Y. F., Reyon, D., Maeder, M. L., Tsai, S. Q., Sander, J. D., Peterson, R. T., et al., (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat. Biotechnol.*, 31, 227–229.
- Inukai, S., Kock, K. H., & Bulyk, M. L., (2017). Transcription factor–DNA binding: Beyond binding site motifs. *Curr. Opin. Genet. Dev.*, 43, 110–119.
- Ishihama, N., & Yoshioka, H., (2012). Post-translational regulation of WRKY transcription factors in plant immunity. *Curr. Opin. Plant Biol.*, 15, 431–437.
- Ito, Y., Katsura, K., Maruyama, K., Taji, T., Kobayashi, M., Seki, S., Shinozaki, K., & Yamaguchi-Shinozaki, K., (2006). Functional analysis of rice DREB1/CBF type transcription factors involved in cold responsive gene expression in transgenic rice. *Plant Cell Physiol.*, 47, 141–153.
- Iturriaga, G., Gaff, G. F., & Zentella, R., (2000). New desiccation tolerant plants, including a grass in the central highlands of Mexico, accumulate trehalose. *Aust. J. Bot.*, 48, 153–158.
- Javed, T., Shabbir, R., Ali, A., Afzal, I., Zaheer, U., & Gao, A. J., (2020). Transcription factors in plant stress responses: Challenges and potential for sugarcane improvement. *Plants*, 9, 491.

- Jazii, R. F., Yamchi, A., Hajirezaei, M., Abbasi, A. R., & Karkhane, A. A., (2011). Growth assessments of *Nicotiana tabacum* cv. xanthi transformed with *Arabidopsis thaliana* P5CS under salt stress. *Afr. J. Biotechnol.*, 10, 8539–8552.
- Jia, D., Jiang, Q., Van, N. S., Gong, X., & Ma, F., (2019). An apple (*Malus domestica*) NAC transcription factor enhances drought tolerance in transgenic apple plants. *Plant Physiol. Biochem.*, 139, 504–512.
- Jiang, C., Xu, J., Zhang, H., Zhang, X., Shi, J., Li, M., & Ming, F., (2009). Acytosolic class I small heat shock protein, RchHSP17.8, of *Rosa chinensis* confers resistance to a variety of stresses to *Escherichia coli*, yeast and *Arabidopsis thaliana*. *Plant Cell Environ.*, 32, 1046–1059.
- Jiang, W. Y., Bikard, D., Cox, D., Zhang, F., & Marraffini, L. A., (2013a). RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nat. Biotechnol.*, 31, 233–239.
- Jiang, W. Z., Zhou, H. B., Bi, H. H., Fromm, M., Yang, B., & Weeks, D. P., (2013b). Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic Acids Res.*, 41, e188.
- Jiang, Y., Duan, Y., Yin, J., Ye, S., Zhu, J., Zhang, F., Lu, W., et al., (2014). Genome-wide identification and characterization of the Populus WRKY transcription factor family and analysis of their expression in response to biotic and abiotic stresses. *J. Exp. Bot.*, 65, 6629–6644.
- Jiang, Y., Liang, G., & Yu, D., (2012). Activated expression of *WRKY57* confers drought tolerance in *Arabidopsis*. *Mol. Plant.*, 5, 1375–1388.
- Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., Charpentier, E., (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337, 816–821.
- Jisha, V., Dampanaboina, L., Vadassery, J., Mithöfer, A., Kappara, S., & Ramanan, R., (2015). Overexpression of an AP2/ERF type transcription factor *OsEREBP1* confers biotic and abiotic stress tolerance in rice. *PLoS One*, 10, e0127831.
- Jyothsnakumari, G., Thippeswamy, M., Veeranagamallaiah, G., & Sudhakar, C., (2009). Differential expression of LEA proteins in two genotypes of mulberry under salinity. *Biol. Plant.*, 53, 145–150.
- Kang, C., Zhai, H., He, S., Zhao, N., & Liu, Q., (2019). A novel sweetpotato bZIP transcription factor gene, *IbbZIP1*, is involved in salt and drought tolerance in transgenic *Arabidopsis*. *Plant Cell Rep.*, 38, 1373–1382.
- Kang, J. Y., Choi, H. I., Im, M. Y., & Kim, S. Y., (2002). *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. *Plant Cell*, 14, 343–357.
- Karim, S., Aronsson, H., Ericson, H., Pirhonen, M., Leyman, B., Welin, B., Mantyla, E., et al., (2007). Improved drought tolerance without undesired side effects in transgenic plants producing trehalose. *Plant Mol. Biol.*, 64, 371–386.
- Karthikeyan, A., Pandian, S. K., & Ramesh, M., (2011). Transgenic indica rice cv. ADT 43 expressing a *Δ1-pyrroline-5-carboxylate synthetase* (P5CS) gene from *Vigna aconitifolia* demonstrates salt tolerance. *Plant Cell Tissue Organ Cult.*, 107, 383–395.
- Kasuga, M., Liu, Q., Miura, S., Yamaguchi, S., & Shinozaki, K., (1999). Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.*, 17, 287–291.
- Kasuga, M., Miura, S., & Yamaguchi-Shinozaki, K., (2004). A combination of the *Arabidopsis* *DREB1A* gene and stress inducible *rd29A* promoter improved drought and low temperature stress tolerance in tobacco by gene transfer. *Plant Cell Physiol.*, 45, 346–350.

- Kathuria, H., Giri, J., Tyagi, H., & Tyagi, A. K., (2007). Advances in transgenic rice biotechnology. *Crit. Rev. Plant Sci.*, 26, 65–103.
- Khan, S. A., Li, M. Z., Wang, S. M., & Yin, H. J., (2019). Revisiting the role of plant transcription factors in the battle against abiotic stress. *Int. J. Mol. Sci.*, 19, 1634.
- Khoshmanzar, E., Aliasgharzad, N., Neyshabouri, M. R., Khoshru, B., Arzanlou, M., & Asgari, L. B., (2020). Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water-deficit stress. *Int. J. Environ. Sci. Technol.*, 17, 869–878.
- Kim, C. Y., Vo, K. T. X., Nguyen, C. D., Jeong, D. H., Lee, S. K., Kumar, M., Kim, S. R., et al., (2016). Functional analysis of a cold-responsive rice WRKY gene, *OsWRKY71*. *Plant Biotechnol. Rep.*, 10, 13–23.
- Kimotho, N., Baillo, E. H., & Zhang, Z., (2019). Transcription factors involved in abiotic stress responses in maize (*Zea mays* L.) and their roles in enhanced productivity in the post genomics era. *Peer J.*, 30, e7211.
- Kishor, P. B. K., Hong, Z., Miao, G. H., Hu, C. A. A., & Verma, D. P. S., (1995). Overexpression of delta 1- pyrroline-5carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol.*, 108, 1387–1394.
- Kitsios, G., & Doonan, J. H., (2011). Cyclin dependent protein kinases and stress responses in plants. *Plant Signal. Behav.*, 6, 204–209.
- Krasensky, J., Broyart, C., Rabanal, F. A., & Jonak, C., (2014). The redox-sensitive chloroplast trehalose-6-phosphate phosphatase AtTPPD regulates salt stress tolerance. *Antioxid Redox Signal*, 21, 1289–1304.
- Kreuzwieser, J., & Rennenberg, H., (2014). Molecular and physiological responses of trees to waterlogging stress. *Plant Cell Environ.*, 37, 2245–2259.
- Kudla, J., Becker, D., Grill, E., Hedrich, R., Hippler, M., Kummer, U., Parniske, M., et al., (2018). Advances and current challenges in calcium signaling. *New Phytol.*, 218, 414–431.
- Kumar, S. G., Reddy, A. M., & Sudhakar, C., (2003). NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. *Plant Sci.*, 165, 1245–1251.
- Kumar, S. V., Sharma, M. L., & Rajam, M. V., (2006). Polyamine biosynthetic pathway as a novel target for potential applications in plant biotechnology. *Physiol. Mol. Biol. Plant.*, 12, 13–28.
- Kumar, V., Shriram, V., Kavi, K. P. B., Jawali, N., & Shitole, M. G., (2010). Enhanced proline accumulation and salt stress tolerance of transgenic indica rice by overexpressing *P5CSF129A* gene. *Plant Biotechnol. Rep.*, 4, 37–48.
- Lamaoui, M., Jemo, M., Datla, R., & Bekkaoui, F., (2018). Heat and drought stresses in crops and approaches for their mitigation. *Front. Chem.*, 6, 26.
- Landi, S., Hausman, J. F., Guerriero, G., & Esposito, S., (2017). Poaceae vs. abiotic stress: Focus on drought and salt stress, recent insights and perspectives. *Front. Plant Sci.*, 8, 1214.
- Langridge, P., Paltridge, N., & Fincher, G., (2006). Functional genomics of abiotic stress tolerance in cereals. *Briefings Funct. Genom. Proteom.*, 4, 343–354.
- Le Hir, R., Castelain, M., Chakraborti, D., Moritz, T., Dinant, S., & Bellini, C., (2017). *AtbHLH68* transcription factor contributes to the regulation of ABA homeostasis and drought stress tolerance in *Arabidopsis thaliana*. *Physiol. Plant.*, 160, 312–327.
- Lee, H. G., & Seo, P. J., (2015). The MYB96-HHP module integrates cold and abscisic acid signaling to activate the CBF-COR pathway in *Arabidopsis*. *Plant J.*, 82, 962–977.
- Lee, S. B., & Suh, M. C., (2015). Cuticular wax biosynthesis is up-regulated by the MYB94 transcription factor in *Arabidopsis*. *Plant Cell Physiol.*, 56, 48–60.

- Lee, S. B., Kim, H. U., & Suh, M. C., (2016). *MYB94* and *MYB96* additively activate cuticular wax biosynthesis in *Arabidopsis*. *Plant Cell Physiol.*, 57, 2300–2311.
- Li, H., Gao, Y., Xu, H., Dai, Y., Deng, D., & Chen, J., (2013). *ZmWRKY33*, a WRKY maize transcription factor conferring enhanced salt stress tolerances in *Arabidopsis*. *Plant Growth Regul.*, 70, 207–216.
- Li, J. F., Norville, J. E., Aach, J., McCormack, M., Zhang, D., Bush, J., Church, G. M., & Sheen, J., (2013). Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nat. Biotechnol.*, 31, 688.
- Li, L., Gao, W., Peng, Q., Zhou, B., Kong, Q., Ying, Y., et al., (2018). Two soybean bHLH factors regulate response to iron deficiency. *J. Integr. Plant Biol.*, 60, 608–622.
- Li, X. W., Wang, Y., Yan, F., Li, J. W., Zhao, Y., Zhao, X., Zhai, Y., & Wang, Q. Y., (2016). Overexpression of soybean R2R3-MYB transcription factor, *GmMYB12B2*, and tolerance to UV radiation and salt stress in transgenic *Arabidopsis*. *Genet. Mol. Res.*, 15, 1–10.
- Li, X., Tang, Y., Li, H., Luo, W., Zhou, C., Zhang, L., et al., (2020). A wheat R2R3 MYB gene *TaMpc1-D4* negatively regulates drought tolerance in transgenic *Arabidopsis* and wheat. *Plant Sci.*, 299, 110613.
- Li, Y., Chen, Q., Nan, H., Li, X., Lu, S., Zhao, X., Liu, B., et al., (2017). Overexpression of *GmFDL19* enhances tolerance to drought and salt stresses in soybean. *PLoS One*, 12, e0179554.
- Li, Y., Zhang, S., Zhang, N., Zhang, W., Li, M., Liu, B., et al., (2019). MYB-CC transcription factor, *TaMYBsm3*, cloned from wheat is involved in drought tolerance. *BMC Plant Biol.*, 19, 1–11.
- Li, Z. X., Liu, C., Zhang, Y., Wang, B. M., Ran, Q. J., & Zhang, J. R., (2019). The bHLH family member *ZmPTF1* regulates drought tolerance in maize by promoting root development and abscisic acid synthesis. *J. Exp. Bot.*, 70, 5471–5486.
- Liang, C., Meng, Z., Meng, Z., Malik, W., Yan, R., Lwin, K. M., Lin, F., et al., (2016). *GhABF2*, a bZIP transcription factor, confers drought and salinity tolerance in cotton (*Gossypium hirsutum* L.). *Sci. Rep.*, 6, 35040.
- Liao, C. C., Zheng, Y., & Guo, Y., (2017). *MYB30* transcription factor regulates oxidative and heat stress responses through ANNEXINs mediated cytosolic calcium signaling in *Arabidopsis*. *New Phytol.*, 216, 163–177.
- Liao, Y., Zou, H. F., Wang, H. W., Zhang, W. K., Ma, B., Zhang, J. S., & Chen, S. Y., (2008). Soybean *GmMYB76*, *GmMYB92* and *GmMYB177* genes confer stress tolerance in transgenic *Arabidopsis* plants. *Cell Res.*, 18, 1047–1060.
- Lindhout, B. I., Pinas, J. E., Hooykaas, P. J., & Van, D. Z. B. J., (2006). Employing libraries of zinc finger artificial transcription factors to screen for homologous recombination mutants in *Arabidopsis*. *Plant J.*, 48, 475–483.
- Liu, C., Mao, B., Ou, S., Wang, W., Liu, L., Wu, Y., et al., (2014). *OsbZIP71*, a bZIP transcription factor, confers salinity and drought tolerance in rice. *Plant Mol. Biol.*, 84, 19–36.
- Liu, C., Sun, Q., Zhao, L., Li, Z., Peng, Z., & Zhang, J., (2018). Heterologous expression of the transcription factor *EsNAC1* in *Arabidopsis* enhances abiotic stress resistance and retards growth by regulating the expression of different target genes. *Front. Plant Sci.*, 9, 1495.



- Liu, D., Chen, X., Liu, J., Ye, J., & Guo, Z., (2012). The rice ERF transcription factor *OsERF922* negatively regulates resistance to *Magnaporthe oryzae* and salt tolerance. *J. Exp. Bot.*, 63, 3899–3911.
- Liu, H., Yang, Y., Liu, D., Wang, X., & Zhang, L., (2020). Transcription factor *TabHLH49* positively regulates dehydrin *WZY2* gene expression and enhances drought stress tolerance in wheat. *BMC Plant Biology*, 20, 259.
- Liu, L., Zhang, Z., Dong, J., & Wang, T., (2016). Overexpression of *MtWRKY76* increases both salt and drought tolerance in *Medicago truncatula*. *Environ. Exp. Bot.*, 123, 50–58.
- Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., & Shinozaki, K., (1998). Two transcription factors, *DREB1* and *DREB2*, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell*, 10, 1391–1406.
- Liu, R., Liu, M., Liu, J., Chen, Y., Chen, Y., & Lu, C., (2010). Heterologous expression of a *Ammopiptanthus mongolicus* late embryogenesis abundant protein gene (*AmLEA*) enhances *Escherichia coli* viability under cold and heat stress. *Plant Growth Regul.*, 60, 163–168.
- Liu, X., Song, Y., Xing, F., Wang, N., Wen, F., & Zhu, C., (2015). *GhWRKY25*, a group I WRKY gene from cotton, confers differential tolerance to abiotic and biotic stresses in transgenic *Nicotiana benthamiana*. *Protoplasma*, 253, 1265–1281.
- Liu, Y., Ji, X., Nie, X., Qu, M., Zheng, L., Tan, Z., Zhao, H., et al., (2015). *Arabidopsis AtbHLH112* regulates the expression of genes involved in abiotic stress tolerance by binding to their E-box and GCG-box motifs. *New Phytol.*, 207, 692–709.
- Liu, Y., Yu, X., Liu, S., Peng, H., Mijiti, A., Wang, Z., Zhang, H., & Ma, H., (2017). A chickpea NAC-type transcription factor, *CarNAC6*, confers enhanced dehydration tolerance in *Arabidopsis*. *Plant Mol. Biol. Rep.*, 35, 83–96.
- Lobell, D. B., Roberts, M. J., Schlenker, W., Braun, N., Little, B. B., Rejesus, R. M., & Hammer, G. L., (2014). Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest. *Science*, 344, 516–519.
- Lowder, L. G., Paul, J. W., Baltes, N. J., Voytas, D. F., Zhang, Y., Zhang, D., Tang, X., et al., (2015). A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. *Plant Physiol.*, 169, 971–985.
- Lowder, L. G., Zhou, J., Zhang, Y., Malzahn, A., Zhong, Z., Hsieh, T. F., Voytas, D. F., et al., (2017). Robust transcriptional activation in plants using multiplexed CRISPR-Act2. 0 and mTALE-act systems. *Mol. Plant*, 11, 245–256.
- Lu, G., Gao, C., Zheng, X., & Han, B., (2009). Identification of *OsbZIP72* as a positive regulator of ABA response and drought tolerance in rice. *Planta*, 229, 605–615.
- Luo, M., Liu, X., Singh, P., Cui, Y., Zimmerli, L., & Wu, K., (2012). Chromatin modifications and remodeling in plant abiotic stress responses. *Biochim. Biophys. Acta*, 1819, 129–136.
- Luo, X., Li, C., He, X., Zhang, X., & Zhu, L. F., (2019). ABA signaling is negatively regulated by *GbWRKY1* through JAZ1 and ABI1 to affect salt and drought tolerance. *Plant Cell Rep.*, 39, 181–194.
- Ma, J., Gao, X., Liu, Q., Shao, Y., Zhang, D., Jiang, L., & Li, C., (2017). Overexpression of *TaWRKY146* increases drought tolerance through inducing stomatal closure in *Arabidopsis thaliana*. *Front. Plant Sci.*, 8, 2036.
- Ma, J., Wang, L. Y., Dai, J. X., Wang, Y., & Lin, D., (2021). The NAC-type transcription factor *CaNAC46* regulates the salt and drought tolerance of transgenic *Arabidopsis thaliana*. *BMC Plant Biol.*, 21, 11.

- Machens, F., Balazadeh, S., Mueller-Roeber, B., & Messerschmidt, K., (2017). Synthetic promoters and transcription factors for heterologous protein expression in *Saccharomyces cerevisiae*. *Front. Bioeng. Biotechnol.*, 5, 63.
- Maeder, M. L., Thibodeau-Beganny, S., Osiak, A., Wright, D. A., et al., (2008). Rapid “open-source” engineering of customized zinc-finger nucleases for highly efficient gene modification. *Mol. Cell*, 31, 294–301.
- Mahboobeh, R., & Akbar, E. A., (2013). Effect of salinity on growth chlorophyll carbohydrate and protein contents of transgenic *Nicotiana plumbaginifolia* over expressing *P5CS* gene. *J. Environ. Res. Manag.*, 4, 163–170.
- Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M., (2013). RNA-guided human genome engineering via Cas9. *Science*, 339, 823–826.
- Manna, M., Thakur, T., Chirom, O., Mandlik, R., Deshmukh, R., & Salvi, P., (2021). Transcription factors as key molecular targets to strengthen the drought stress tolerance in plants. *Physiol. Plant.*, 172, 847–868.
- Mao, H., Li, S., Wang, Z., Cheng, X., Li, F., Mei, F., Chen, N., & Kang, Z., (2019). Regulatory changes in *TaSNAC8-6A* are associated with drought tolerance in wheat seedlings. *Plant Biotechnol. J.*, 18, 1078–1092.
- Mao, H., Yu, L., Han, R., Li, Z., & Liu, H., (2016). *ZmNAC55*, a maize stress-responsive NAC transcription factor, confers drought resistance in transgenic *Arabidopsis*. *Plant Physiol. Biochem.*, 105, 55–66.
- McNeil, S. D., Nuccio, M. L., & Hanson, A. D., (1999). Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. *Plant Physiol.*, 120, 945–949.
- McNeil, S. D., Nuccio, M. L., Rhodes, D., Shachar-Hill, Y., & Hanson, A. D., (2000). Radiotracer and computer modeling evidence that phosphobase methylation in the main route of choline synthesis in tobacco. *Plant Physiol.*, 123, 371–380.
- McNeil, S. D., Nuccio, M. L., Ziemak, M. J., & Hanson, A. D., (2001). Enhanced synthesis of choline and glycine betaine in transgenic tobacco plants that overexpress phosphoethanolamine N-methyltransferase. *Proc. Natl. Acad. Sci. USA.*, 98, 10001–10005.
- Meng, X., Wang, J. R., Wang, G. D., Liang, X. Q., Li, X. D., & Meng, Q. W., (2015). An R2R3-MYB gene, *LeAN2*, positively regulated the thermo-tolerance in transgenic tomato. *J. Plant Physiol.*, 175, 1–8.
- Meng, X., Yin, B., Feng, H. L., Zhang, S., Liang, X. Q., & Meng, Q. W., (2014). Overexpression of R2R3-MYB gene leads to accumulation of anthocyanin and enhanced resistance to chilling and oxidative stress. *Biol. Plant.*, 58, 121–130.
- Merchuk-Ovnat, L., Barak, V., Fahima, T., Ordon, F., Lidzbarsky, G. A., Krugman, T., & Saranga, Y., (2016). Ancestral QTL alleles from wild emmer wheat improve drought resistance and productivity in modern wheat cultivars. *Front. Plant Sci.*, 7, 452.
- Miao, J., Guo, D. S., Zhang, J. Z., Huang, Q. P., Qin, G. J., Zhang, X., Wan, J. M., et al., (2013). Targeted mutagenesis in rice using CRISPR-Cas system. *Cell Res.*, 23, 1233–1236.
- Misr, S., Wu, Y., Venkataraman, G., Sopory, S. K., & Tuteja, N., (2007). Heterotrimeric G-protein complex and G-protein-coupled receptor from a legume (*Pisum sativum*): Role in salinity and heat stress and cross-talk with phospholipase C. *Plant J.*, 51, 656–669.
- Mitsuda, N., Matsui, K., Ikeda, M., Nakata, M., Oshima, Y., Nagatoshi, Y., & Ohme-Takagi, M., (2011). CRES-T, an effective gene silencing system utilizing chimeric repressors. *Methods Mol. Biol.*, 754, 87–105.
- Mittler, R., & Blumwald, E., (2010). Genetic engineering for modern agriculture: Challenges and perspectives. *Annu. Rev. of Plant Biol.*, 61, 443–462.

- Molinari, H. B. C., Marur, C. J., Daros, E., De Campos, M. K. F., Rodrigues, J. F., De Carvalho, P., Filho, J. C. B., et al., (2007). Evaluation of the stress-inducible production of proline in transgenic sugarcane (*Saccharum* spp.): Osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Plant Physiol.*, 130, 218–229.
- Moon, S. J., Han, S. Y., Kim, D. Y., Yoon, I. S., Shin, D., Byun, M. O., Kwon, H. B., & Kim, B. G., (2015). Ectopic expression of a hot pepper bZIP-like transcription factor in potato enhances drought tolerance without decreasing tuber yield. *Plant Mol. Biol.*, 89, 421–431.
- Morran, S., Eini, O., Pyvovarenko, T., Parent, B., Singh, R., Ismagul, A., Eilby, S., et al., (2011). Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. *Plant Biotechnol J.*, 9, 230–249.
- Naidu, B. P., Paleg, L. G., Aspinall, D., Jennings, A. C., & Jones, G. P., (1991). Amino acid and glycine betaine accumulation in cold stressed wheat seedlings. *Phytochemistry*, 30, 407–409.
- Nakashima, K., Fujita, Y., Katsura, K., Maruyama, K., Narusaka, Y., Seki, M., et al., (2006). Transcriptional regulation of *ABI3* and ABA-responsive genes including *RD29B* and *RD29A* in seeds, germinating embryos, and seedlings of *Arabidopsis*. *Plant Mol. Biol.*, 60, 51–68.
- Nakashima, K., Tran, L. S., Van, N. D., Fujita, M., Maruyama, K., Todaka, D., Ito, Y., et al., (2007). Functional analysis of a NAC-type transcription factor *OsNAC6* involved in abiotic and biotic stress-responsive gene expression in rice. *Plant J.*, 51, 617–630.
- Nekrasov, V., Staskawicz, B., Weigel, D., Jones, J. D. G., & Kamoun, S., (2013). Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nat. Biotechnol.*, 31, 691–693.
- Nelson, D. E., Repetti, P. P., Adams, T. R., Creelman, R. A., Wu, J., Warner, D. C., Anstrom, D. C., et al., (2007). Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proc. Natl. Acad. Sci., USA.*, 104, 16450–16455.
- Nemhauser, J. L., Hong, F., & Chory, J., (2006). Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell*, 126, 467–475.
- Noman, M., Aysha, J., Keteouli, T., Yang, J., Du, L., Wang, F., & Li, H., (2021). Calmodulin binding transcription activators: An interplay between calcium signaling and plant stress tolerance. *J. Plant Physiol.*, 256, 153327.
- Nomura, M., Muramoto, Y., Yasuda, S., Takabe, T., & Kishitani, S., (1995). The accumulation of glycinebetaine during cold acclimation in early and late cultivars of barley. *Euphytica*, 83, 247–250.
- Novillo, F., Alonso, J. M., Ecker, J. R., & Salinas, J., (2004). *CBF2/DREB1C* is a negative regulator of *CBF1/DREB1B* and *CBF3/DREB1A* expression and plays a central role in stress tolerance in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA.*, 101, 3985–3990.
- Nuccio, M. L., Rhodes, D., McNeil, S. D., & Hanson, A. D., (1999). Metabolic engineering of plants for osmotic stress resistance. *Curr. Opin. Plant Biol.*, 2, 128–134.
- Nuccio, M. L., Russell, B. L., Nolte, K. D., Rathinasabapathi, B., Gage, D. A., & Hanson, A. D., (1998). The endogenous choline supply limits glycine betaine synthesis in transgenic tobacco expressing *choline monoxygenase*. *Plant J.*, 16, 487–496.
- Nuccio, M. L., Wu, J., Mowers, R., Zhou, H. P., Meghji, M., Primavesi, L. F., Paul, M. J., Chen, X., Gao, Y., Haque, E., et al., (2015). Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. *Nat. Biotechnol.*, 33, 862–869.

- Nuruzzaman, M., Sharoni, A. M., & Kikuchi, S., (2013). Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. *Front. Microbiol.*, 4, 248.
- Oh, S. J., Kwon, C. W., Choi, D. W., Song, S. I. K., & Kim, J. K., (2007). Expression of barley *HvCBF4* enhances tolerance to abiotic stress in transgenic rice. *J. Plant Biotechnol.*, 5, 646–656.
- Oh, S. J., Song, S. I., Kim, Y. S., Jang, H. J., Kim, M., & Kim, Y. K., (2005). *Arabidopsis* *CBF3/DREB1A* and *ABF3* in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiol.*, 138, 341–351.
- Pan, Y., Hu, X., Li, C., Xu, X., Su, C., Li, J., Song, H., Zhang, X., & Pan, Y., (2017). *SlbZIP38*, a tomato bZIP family gene down regulated by abscisic acid, is a negative regulator of drought and salt stress tolerance. *Genes*, 8, 402.
- Pandey, A. S., Sharma, E., Jain, N., Singh, B., Burman, N., & Khurana, J. P., (2018). A rice bZIP transcription factor, *OsbZIP16*, regulates abiotic stress tolerance when overexpressed in *Arabidopsis*. *J. Plant Biochem. Biotechnol.*, 27, 393–400.
- Pandey, S. P., & Somssich, I. E., (2009). The role of WRKY transcription factors in plant immunity. *Plant Physiol.*, 150, 1648–1655.
- Papageorgiou, G. C., & Murata, N., (1995). The unusually strong stabilizing effects of glycinebetaine on the structure and function of oxygen-evolving photosystem II complex. *Photosynth Res.*, 44, 243–252.
- Park, E. J., Jeknic, Z., Pino, M. T., Murata, N., & Chen, T. H. H., (2007). Glycine betaine accumulation is more effective in chloroplasts than in the cytosol for protecting transgenic tomato plants against abiotic stress. *Plant Cell Environ.*, 30, 994–1005.
- Paul, J. M., Primavesi, L. F., Jhurrea, D., & Zhang, Y., (2008). Trehalose metabolism and signaling. *Annu. Rev. Plant Biol.*, 59, 417–441.
- Peleg, Z., & Blumwald, R., (2011). Hormone balance and abiotic stress tolerance in crop plants. *Curr Opin. Plant Biol.*, 14, 290–295.
- Pellegrineschi, A., Reynolds, M., Pacheco, M., Brito, R. M., Almeraya, R., Yamaguchi-Shinozaki, K., & Hoisington, D., (2004). Stress induced expression in wheat of the *Arabidopsis thaliana* *DREB1A* gene delays water stress symptoms under greenhouse conditions. *Genome.*, 47, 493–500.
- Puranik, S., Sahu, P. P., Srivastava, P. S., & Prasad, M., (2012). NAC proteins: Regulation and role in stress tolerance. *Trends Plant Sci.*, 17, 369–381.
- Qin, F., Kakimoto, M., Sakuma, Y., Maruyama, K., Osakabe, Y., Tran, L. S. P., Shinozaki, K., & Yamaguchi-Shinozaki, K., (2007). Regulation and functional analysis of *ZmDREB2A* in response to drought and heat stresses in *Zea mays* L. *Plant J.*, 50, 54–69.
- Qin, Z., Hou, F., Li, A., Dong, S., Wang, Q., & Zhang, L., (2020). Transcriptome-wide identification of WRKY transcription factor and their expression profiles under salt stress in sweet potato (*Ipomoea batatas* L.). *Plant Biotechnol. Rep.*, 14, 599–611.
- Qiu, Y., & Yu, D., (2009). Over-expression of the stress induced *OsWRKY45* enhances disease resistance and drought tolerance in *Arabidopsis*. *Environ. Exp. Bot.*, 65, 35–47.
- Qiu, Z., Wang, X., Gao, J., Guo, Y., Huang, Z., & Du, Y., (2016). The tomato Hoffman's anthocyaninless gene encodes a bHLH transcription factor involved in anthocyanin biosynthesis that is developmentally regulated and induced by low temperatures. *PLoS One*, 11, e0151067.
- Quan, R., Hu, S., Zhang, Z., Zhang, H., Zhang, Z., & Huang, R., (2010). Overexpression of an ERF transcription factor *TSRF1* improves rice drought tolerance. *Plant Biotechnol. J.*, 8, 476–488.

- Rahman, H., Ramanathan, V., Nallathambi, J., Duraialagaraja, S., & Muthurajan, R., (2016). Over-expression of a *NAC67* transcription factor from finger millet (*Eleusine coracana* L.) confers tolerance against salinity and drought stress in rice. *BMC Biotechnol.*, 16, 7–20.
- Raineri, J., Wang, S., Peleg, Z., Blumwald, E., & Chan, R. L., (2015). The rice transcription factor *OsWRKY47* is a positive regulator of the response to water deficit stress. *Plant Mol. Biol.*, 88, 401–413.
- Rajam, M. V., Dagar, S., Waie, B., Yadav, J. S., Kumar, P. A., Shoeb, F., & Kumria, R., (1998). Genetic engineering of polyamine and carbohydrate metabolism for osmotic stress tolerance in higher plants. *J. Biosci.*, 23, 473–482.
- Ramalingam, A., Kudapa, H., Pazhamala, L. T., Garg, V., & Varshney, R. K., (2015). Gene expression and yeast two-hybrid studies of *IR-MYB* transcription factor mediating drought stress response in chickpea (*Cicer arietinum* L.). *Front. Plant Sci.*, 6, 1117.
- Ray, D. K., Ramankutty, N., Mueller, N. D., West, P. C., & Foley, J. A., (2012). Recent patterns of crop yield growth and stagnation. *Nat. Commun.*, 3, 1293.
- Reddy, P. S., Jogeswar, G., Rasineni, G. K., Maheswari, M., Reddy, A. R., & Varshney, R. K., (2015). Proline over-accumulation alleviates salt stress and protects photosynthetic and antioxidant enzyme activities in transgenic sorghum (*Sorghum bicolor* (L.) Moench). *Plant Physiol Biochem.*, 94, 104–113.
- Reis, R. R., Da Cunha, B. A., Martins, P. K., Martins, M. T., Alekcevetch, J. C., & Chalfun-Junior, A., (2014). Induced overexpression of *AtDREB2A* CA improves drought tolerance in sugarcane. *Plant Sci.*, 221, 222, 59–68.
- Richards, R. A., (1996). Defining selection criteria to improve yield under drought. *Plant Growth Regul.*, 20, 157–166.
- Riechmann, J. L., Heard, J., Martin, G., Reuber, L., Jiang, C. Z., Keddie, J., Adam, L., et al., (2000). *Arabidopsis* transcription factors: Genome-wide comparative analysis among eukaryotes. *Science*, 290, 2105–2110.
- Rushton, P. J., Somssich, I. E., Ringler, P., & Shen, Q. J., (2010). WRKY transcription factors. *Trends Plant Sci.*, 15, 247–258.
- Saad, A. S. I., Li, X., Li, H. P., Huang, T., Gao, C. S., Guo, M. W., & Liao, Y. C., (2013). A rice stress-responsive NAC gene enhances tolerance of transgenic wheat to drought and salt stresses. *Plant Sci.*, 203, 204, 33–40.
- Sakamoto, A., & Murata, N., (2001). The use of choline oxidase, a glycinebetaine-synthesizing enzyme, to create stress resistant transgenic plants. *Plant Physiol.*, 125, 180–188.
- Sakuma, Y., Maryyama, K., Qin, F., Osakabe, Y., Shinozaki, K., & Yamaguchi-Shinozaki, K., (2006). Dual function of an *Arabidopsis* transcription factor *DREB2A* in water-stress-responsive and heat-stress-responsive gene expression. *Proc. Natl. Acad. Sci. USA.*, 103, 18822–18827.
- Samira, R., Li, B., Kliebenstein, D., Li, C., Davis, E., Gillikin, J. W., & Long, T. A., (2018). The bHLH transcription factor *ILR3* modulates multiple stress responses in *Arabidopsis*. *Plant Mol. Biol.*, 97, 297–309.
- Sato, Y., & Yokoya, S., (2008). Enhanced tolerance to drought in transgenic rice plants overexpressing a small heat-shock protein, sHSP17.7. *Plant Cell Rep.*, 27, 329–334.
- Schaller, G. E., Kieber, J. J., & Shiu, S. H., (2008). Two-component signaling elements and histidyl-aspartyl phosphorelays. In: *The Arabidopsis Book*. The American Society of Plant Biologists.

- Schmidt, R., Mieulet, D., Hubberten, H. M., Obata, T., Hoefgen, R., Fernie, A. R., Fisahn, J., et al., (2013). Salt-responsive ERF1 regulates reactive oxygen species-dependent signaling during the initial response to salt stress in rice. *Plant Cell*, 25, 2115–2131.
- Schramm, F., Larkindale, J., Kiehlmann, E., Ganguli, A., English, G., Vierling, E., & Von Koskull-Doring, P. A., (2008). Cascade of transcription factor *DREB2A* and heat stress transcription factor *HsfA3* regulates the heat stress response of *Arabidopsis*. *Plant J.*, 53, 264–274.
- Schulz, P., Herde, M., & Romeis, T., (2013). Calcium-dependent protein kinases: Hubs in plant stress signaling and development. *Plant Physiol.*, 163, 523–530.
- Schwartz, A. R., Morbitzer, R., Lahaye, T., & Staskawicz, B. J., (2017). TALE-induced bHLH transcription factors that activate a pectate lyase contribute to water soaking in bacterial spot of tomato. *Proc. Natl. Acad. Sci. USA*, 114, E897–E903.
- Seki, M., Narusaka, M., Abe, M., Kasuga, M., Yamaguchi-Shinozaki, K., Carninci, P., Hayashizaki, Y., & Shinozaki, K., (2001). Monitoring the expression pattern of 1300 *Arabidopsis* under drought and cold stresses by using a full-length cDNA microarray. *Plant Cell*, 13, 62–72.
- Seki, M., Umezawa, T., Urano, K., & Shinozaki, K., (2007). Regulatory metabolic networks in drought stress responses. *Curr. Opin. Plant Biol.*, 10, 296–302.
- Seo, J. S., Sohn, H. B., Noh, K., Jung, C., An, J. H., Donovan, C. M., Somers, D. A., et al., (2012). Expression of the *Arabidopsis AtMYB44* gene confers drought/salt-stress tolerance in transgenic soybean. *Mol. Breed.*, 29, 601–608.
- Serraj, R., & Sinclair, T. R., (2002). Osmolyte accumulation: Can it really help increase crop yield under drought conditions? *Plant Cell Environ.*, 25, 333–341.
- Shan, H., Chen, S., Jiang, J., Chen, F., Chen, Y., Gu, C., et al., (2012). Heterologous expression of the *Chrysanthemum* R2R3-MYB transcription factor *CmMYB2* enhances drought and salinity tolerance, increases hypersensitivity to ABA and delays flowering in *Arabidopsis thaliana*. *Mol. Biotechnol.*, 51, 160–173.
- Shan, Q. W., Wang, Y. P., Li, J., Zhang, Y., Chen, K. L., Liang, Z., Zhang, K., et al., (2013). Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat. Biotechnol.*, 31, 686–688.
- Shang, Y., Yan, L., Liu, Z. Q., Cao, Z., Mei, C., Xin, Q., et al., (2010). The mg-chelataase H subunit of *Arabidopsis* antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition. *Plant Cell*, 22, 1909–1935.
- Shen, X., Guo, X., Guo, X., Zhao, D., Zhao, W., Chen, J., & Li, T., (2017). *PacMYBA*, a sweet cherry R2R3-MYB transcription factor, is a positive regulator of salt stress tolerance and pathogen resistance. *Plant Physiol. Biochem.*, 112, 302–311.
- Sheveleva, E. V., Marquez, S., Chmara, W., Zegeer, A., Jensen, R. G., & Bohnert, H. J., (1998). Sorbitol-6-phosphate dehydrogenase expression in transgenic tobacco. High amounts of sorbitol lead to necrotic lesions. *Plant Physiol.*, 117, 831–839.
- Shih, M. D., Lin, S. C., Hsieh, J. S., Tsou, C. H., Chow, T. Y., Lin, T. P., & Hsing, Y. I. C., (2004). Gene cloning and characterization of a soybean (*Glycine max* L.) LEA protein, GmPM16. *Plant Mol. Biol.*, 56, 689–703.
- Shim, J. S., Oh, N., Chung, P. J., Kim, Y. S., Choi, Y. D., & Kim, J., (2018). Overexpression of *OsNAC14* improves drought tolerance in rice. *Front. Plant Sci.*, 9, 1–14.
- Shinde, H., Dudhate, A., Tsugama, D., Gupta, S. K., Liu, S., & Takano, T., (2019). Pearl millet stress-responsive NAC transcription factor *PgNAC21* enhances salinity stress tolerance in *Arabidopsis*. *Plant Physiol. Biochem.*, 135, 546–553.

- Shrestha, A., Khan, A., & Dey, N., (2014). Cis-trans engineering: Advances and perspectives on customized transcriptional regulation in plants. *Mol. Plant.*, 11, 886–898.
- Shrivastava, P., & Kumar, R., (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.*, 22, 123–131.
- Singer, M. A., & Lindquist, S., (1998). Thermotolerance in *Saccharomyces cerevisiae*: The yin and yang of trehalose. *Trends Biotechnol.*, 16, 460–468.
- Singh, A. K., Sharma, V., Pal, A. K., Acharya, V., & Ahuja, P. S., (2013). Genome-wide organization and expression profiling of the NAC transcription factor family in potato (*Solanum tuberosum* L.). *DNA Res.*, 20, 403–423.
- Singh, K., Foley, R. C., & Oñate-Sánchez, L., (2002). Transcription factors in plant defense and stress responses. *Curr. Opin. Plant Biol.*, 5, 430–436.
- Smart, C. C., & Flores, S., (1997). Overexpression of *D-myo-inositol-3-phosphate synthase* leads to elevated levels of inositol in *Arabidopsis*. *Plant Mol. Biol.*, 33, 811–820.
- So, H., & Lee, J., (2019). NAC transcription factors from soybean (*Glycine max* L.) differentially regulated by abiotic stress. *J. Plant Biol.*, 62, 147–160.
- Stiller, I., Dulai, S., Kondrák, M., Tarnai, R., Szabó, L., Toldó, O., & Bánfalvi, Z., (2008). Effects of drought on water content and photosynthetic parameters in potato plants expressing the trehalose-6-phosphate synthase gene of *Saccharomyces cerevisiae*. *Planta*, 227, 299–308.
- Su, J., Hirji, R., Zhang, L., He, C., Selvaraj, G., & Wu, R., (2006). Evaluation of the stress-inducible production of *choline oxidase* in transgenic rice as a strategy for producing the stress-protectant glycinebetaine. *J. Exp. Bot.*, 57, 1129–1135.
- Suárez, R., Calderón, C., & Iturriaga, G., (2009). Enhanced tolerance to multiple abiotic stresses in transgenic alfalfa accumulating trehalose. *Crop Sci.*, 49, 1791–1799.
- Sun, J., Hu, W., Zhou, R., Wang, L., Wang, X., Wang, Q., Feng, Z. J., et al., (2014). The *Brachypodium distachyon* *BdWRKY36* gene confers tolerance to drought stress in transgenic tobacco plants. *Plant Cell Rep.*, 34, 23–35.
- Sun, P., Zhu, X., Huang, X., & Liu, J. H., (2014). Overexpression of a stress-responsive MYB transcription factor of *Poncirus trifoliata* confers enhanced dehydration tolerance and increases polyamine biosynthesis. *Plant Physiol. Biochem.*, 78, 71–77.
- Sun, Y., & Yu, D., (2015). Activated expression of *AtWRKY53* negatively regulates drought tolerance by mediating stomatal movement. *Plant Cell Rep.*, 34, 1295–1306.
- Sun, Y., Zhao, J., Li, X., & Li, Y., (2020). E2 conjugases UBC1 and UBC2 regulate MYB42-mediated SOS pathway in response to salt stress in *Arabidopsis*. *New Phytol.*, 227, 455–472.
- Suzuki, N., Bajad, S., Shuman, J., Shulaev, V., & Mittler, R., (2008). The transcriptional co-activator *mbf1c* is a key regulator of the thermotolerance in *Arabidopsis thaliana*. *J. Biol. Chem.*, 283, 9269–9275.
- Suzuki, N., Miller, G., Salazar, C., Mondal, H. A., Shulaev, E., Cortes, D. F., Shuman, J. L., et al., (2013). Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants. *Plant Cell*, 25, 3553–3569.
- Tang, G. Y., Shao, F. X., Xu, P. L., Shan, L., & Liu, Z. J., (2017). Over-expression of a peanut NAC Gene, *AhNAC4*, confers enhanced drought tolerance in tobacco. *Rus. J. Plant Physiol.*, 64, 525–535.
- Tang, Y., Bao, X., Zhi, Y., Wu, Q., Guo, Y., Yin, X., Zeng, L., Li, J., Zhang, J., He, W., et al., (2019). Overexpression of a MYB family gene, *OsMYB6*, increases drought and salinity stress tolerance in transgenic rice. *Front. Plant Sci.*, 10, 168.

- Tardieu, F., (2012). Any trait or trait-related allele can confer drought tolerance: Just design the right drought scenario. *J. Exp. Bot.*, 63, 25–31.
- Thap, G., Dey, M., Sahoo, L., & Panda, S., (2011). An insight into the drought stress induced alterations in plants. *Biol. Plant.*, 55, 603–613.
- Theocharis, A., Clément, C., & Barka, E. A., (2012). Physiological and molecular changes in plants grown at low temperatures. *Planta*, 235, 1091–1105.
- Thippeswamy, M., Chandraobulreddy, P., Sinilal, B., Shivakumar, M., & Sudhakar, C., (2010). Proline accumulation and the expression of *D1-pyrroline-5-carboxylate synthase* in two safflower cultivars. *Biol. Plant.*, 54, 386–390.
- Thirumalaikumar, V. P., Devkar, V., Mehterov, N., Ali, S., Ozgur, R., Turkan, I., Mueller-Roeber, B., & Balazadeh, S., (2018). NAC transcription factor *JUNGBRUNNEN 1* enhances drought tolerance in tomato. *Plant Biotechnol. J.*, 16, 354–366.
- Thomas, J. C., Sepahi, M., Arendall, B., & Bohnert, H. J., (1995). Enhancement of seed germination in high salinity by engineering mannitol expression in *Arabidopsis thaliana*. *Plant Cell Environ.*, 18, 801–806.
- Todaka, D., Nakashima, K., Maruyama, K., Kidokoro, S., Osakabe, Y., et al., (2012). Rice phytochrome-interacting factor-like protein *OsPIL1* functions as a key regulator of internode elongation and induces a morphological response to drought stress. *Proc. Natl. Acad. Sci. USA*, 109, 15947–15952.
- Török, Z., Goloubinoff, P., Horvath, I., Tsvetkova, N. M., Glatz, A., Balogh, G., Varvasovszki, V., et al., (2001). Synechocystis HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone mediated refolding. *Proc. Nat. Acad. Sci. USA*, 98, 3098–3103.
- Townsend, J. A., Wright, D. A., Winfrey, R. J., Fu, F., Maeder, M. L., Joung, J. K., & Voytas, D. F., (2009). High-frequency modification of plant genes using engineered zinc-finger nucleases. *Nature*, 459, 442.
- Tran, L. S., Nakashima, K., Sakuma, Y., Simpson, S. D., Fujita, Y., Maruyama, K., Fujita, M., et al., (2004). Isolation and functional analysis of *Arabidopsis* stress inducible NAC transcription factors that bind to a drought responsive cis-element in the early responsive to dehydration stress 1 promoter. *Plant Cell*, 16, 2481–2498.
- Trujillo, L., Menendez, C., Ochogavia, M. E., Hernandez, I., Borrás, O., Rodriguez, R., Coll, Y., et al., (2009). Engineering drought and salt tolerance in plants using *SodERF3*, a novel sugarcane ethylene responsive factor. *Biotechnol. Appl.*, 26, 168–171.
- Ullah, A., Sun, H., Yang, X., & Zhang, X., (2017). A novel cotton WRKY gene, *GhWRKY6*-like, improves salt tolerance by activating the ABA signaling pathway and scavenging of reactive oxygen species. *Physiol. Plant.*, 162, 439–454.
- Valliyodan, B., & Nguyen, H., (2006). Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Curr. Opin. Plant Biol.*, 9, 1–7.
- Van Ha, C., Esfahani, M. N., Watanabe, Y., Tran, U. T., Sulieman, S., Mochida, K., Van, N. D., & Tran, L. S. P., (2014). Genome-wide identification and expression analysis of the *CaNAC* family members in chickpea during development, dehydration and ABA treatments. *PLoS One*, 9, e114107.
- Van, T. N., Rolloos, M., Pinas, J. E., Henkel, C. V., Augustijn, D., Hooykaas, P. J., & Van, D. Z. B. J., (2017). Enhancement of *Arabidopsis* growth characteristics using genome interrogation with artificial transcription factors. *PLoS One*, 12, e0174236.



- Vendruscolo, E. C. G., Schuster, I., Pileggi, M., Scapim, C. A., Molinari, H. B. C., Marur, C. J., & Vieira, L. G. E., (2007). Stress induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant Physiol.*, 164, 1367–1376.
- Vierling, E., (1991). The roles of heat-shock proteins in plants. *Ann. Rev. Plant Biol.*, 42, 579–620.
- Villalobos, M. A., Bartels, B., & turriaga, G., (2004). Stress tolerance and glucose insensitive phenotypes in *Arabidopsis* overexpressing the *CpMYB10* transcription factor gene1. *Plant Physiol.*, 135, 309–324.
- Vinocur, B., & Altman, A., (2005). Recent advances in engineering plant tolerance to abiotic stress: Achievements and limitations. *Curr. Opin. Biotechnol.*, 16, 123–132.
- Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R. K., Kumar, V., et al., (2017). Abscisic acid signaling and abiotic stress tolerance in plants: A review on current knowledge and future prospects. *Front. Plant Sci.*, 8, 161.
- Vogel, M. O., Moore, M., König, K., Pecher, P., Alsharafa, K., Lee, J., & Dietz, K. J., (2014). Fast retrograde signaling in response to high light involves metabolite export, mitogen-activated protein kinase6, and AP2/ERF transcription factors in *Arabidopsis*. *Plant Cell*, 26, 1151–1165.
- Volkov, R. A., Panchuk, I. I., Mullineaux, P. M., & Schoffl, F., (2006). Heat stress-induced  $H_2O_2$  is required for effective expression of heat shock genes in *Arabidopsis*. *Plant Mol. Biol.*, 61, 733–746.
- Wang, C. T., Ru, J. N., Liu, Y. W., Yang, J. F., Li, M., Xu, Z. S., & Fu, J. D., (2018). The maize WRKY transcription factor *ZmWRKY40* confers drought resistance in transgenic *Arabidopsis*. *Int. J. Mol. Sci.*, 19, 2580.
- Wang, C., Lu, G., Hao, Y., Guo, H., Guo, Y., Zhao, J., & Cheng, H., (2017). *ABP9*, a maize bZIP transcription factor, enhances tolerance to salt and drought in transgenic cotton. *Planta*, 246, 453–469.
- Wang, F., Chen, H. W., Li, Q. T., Wei, W., Li, W., Zhang, W. K., Ma, B., et al., (2015). GmWRKY27 interacts with GmMYB174 to reduce expression of *GmNAC29* for stress tolerance in soybean plants. *Plant J.*, 83, 224–236.
- Wang, F., Kong, W., Wong, G., Fu, L., Peng, R., Li, Z., & Yao, Q., (2016). *AtMYB12* regulates flavonoids accumulation and abiotic stress tolerance in transgenic *Arabidopsis thaliana*. *Mol. Genet. Genom.*, 291, 1545–1559.
- Wang, G., Zhang, S., Ma, X., Wang, Y., Kong, F., & Meng, Q., (2016). A stress-associated NAC transcription factor (*SINAC35*) from tomato plays a positive role in biotic and abiotic stresses. *Physiol. Plant.*, 158, 45–64.
- Wang, J., Lian, W., Cao, Y., Wang, X., Wang, G., Qi, C., Liu, L., et al., (2018). Overexpression of *BoNAC019*, a NAC transcription factor from *Brassica oleracea*, negatively regulates the dehydration response and anthocyanin biosynthesis in *Arabidopsis*. *Sci. Rep.*, 8, 1–15.
- Wang, J., Wang, L., Yan, Y., Zhang, S., Li, H., Gao, Z., Wang, C., & Guo, X., (2020). *GhWRKY21* regulates ABA-mediated drought tolerance by fine-tuning the expression of *GhHAB* in cotton. *Plant Cell Rep.*, 39. [10.1007/s00299-020-02590-4](https://doi.org/10.1007/s00299-020-02590-4).
- Wang, J., Zhou, J., Zhang, B., Vanitha, J., Ramachandran, S., & Jiang, S. Y., (2011). Genome-wide expansion and expression divergence of the basic leucine zipper transcription factors in higher plants with an emphasis on sorghum. *J. Integr. Plant Biol.*, 53, 212–231.
- Wang, L., Ma, H., & Lin, J., (2019). Angiosperm-wide and family-level analyses of AP2/ERF genes reveal differential retention and sequence divergence after whole-genome duplication. *Front. Plant Sci.*, 10, 196.

- Wang, M. Q., Huang, Q. X., Lin, P., Zeng, Q. H., Li, Y., Liu, Q. L., Zhang, L., et al., (2020). The overexpression of a transcription factor gene *VbWRKY32* enhances the cold tolerance in *Verbena bonariensis*. *Front. Plant Sci.*, 10, 1746.
- Wang, N. N., Xu, S. W., Sun, Y. L., Liu, D., Zhou, L., Li, Y., et al., (2019). The cotton WRKY transcription factor (*GhWRKY33*) reduces transgenic *Arabidopsis* resistance to drought stress. *Sci. Rep.*, 9, 1–13.
- Wang, N., Zhang, W., Qin, M., Li, S., Qiao, M., Liu, Z., & Xiang, F., (2017). Drought tolerance conferred in soybean (*Glycine max*. L.) by *GmMYB84*, a novel R2R3-MYB transcription factor. *Plant Cell Physiol.*, 58, 1764–1776.
- Wang, Q. Y., Guan, Y. C., Wu, Y. R., Chen, H. L., Chen, F., & Chu, C. C., (2008). Overexpression of a rice *OsDREB1F* gene increases salt, drought and low temperature tolerance in both *Arabidopsis* and rice. *Plant Mol. Biol.*, 67, 589–602.
- Wang, W. X., Vinocur, B., & Altman, A., (2003). Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, 219, 1–14.
- Wang, W., Qiu, X., Yang, Y., Kim, H. S., Jia, X., Yu, H., et al., (2019). Sweet potato bZIP transcription factor *IbABF4* confers tolerance to multiple abiotic stresses. *Front. Plant Sci.*, 10, 630.
- Wang, Z., Su, G., Li, M., Ke, Q., Kim, S. Y., Li, H., Huang, J., et al., (2016). Overexpressing *Arabidopsis ABF3* increases tolerance to multiple abiotic stresses and reduces leaf size in alfalfa. *Plant Physiol. Biochem.*, 109, 199–208.
- Wani, S. H., Dutta, T., Neelapu, N. R. R., & Surekha, C., (2017). Transgenic approaches to enhance salt and drought tolerance in plants. *Plant Gene.*, 11, 219–231.
- Waqas, M., Azhar, M. T., Rana, I. A., Azeem, F., Ali, M. A., Nawaz, M. A., Chuang, G., & Atif, R. M., (2019). Genome-wide identification and expression analyses of WRKY transcription factor family members from chickpea (*Cicer arietinum* L.) reveal their role in abiotic stress responses. *Gene Genom.*, 41, 467–481.
- Waters, E. R., Lee, G. J., & Vierling, E., (1996). Evolution, structure and function of the small heat shock proteins in plants. *J. exp. Bot.*, 47, 325–338.
- Weber, W., & Fussenegger, M., (2011). Molecular diversity: The toolbox for synthetic gene switches and networks. *Curr. Opin. Chem. Biol.*, 15, 414–420.
- Wiedenheft, B., Sternberg, S. H., & Doudna, J. A., (2012). RNA-guided genetic silencing systems in bacteria and archaea. *Nature*, 482, 331.
- Wu, X., Shiroto, Y., Kishitani, S., Ito, Y., & Toriyama, K., (2009). Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing *OsWRKY11* under the control of HSP101 promoter. *Plant Cell Rep.*, 28, 21–30.
- Wyn, J. R. G., (1984). Phytochemical aspects of osmotic adaptation. *Rec. Adv. Phytochem.*, 18, 55–78.
- Wyvekens, N., Topkar, V. V., Khayter, C., Joung, J. K., & Tsai, S. Q., (2015). Dimeric CRISPR RNA-guided FokI-dCas9 nucleases directed by truncated gRNAs for highly specific genome editing. *Hum. Gene Ther.*, 26, 425–431.
- Xiang, Y., Tang, N., Du, H., Ye, H., & Xiong, L., (2008). Characterization of *OsZIP23* as a key player of the basic leucine zipper transcription factor family for conferring abscisic acid sensitivity and salinity and drought tolerance in rice. *Plant Physiol.*, 148, 1938–1952.
- Xie, Y., Chen, P., Yan, Y., Bao, C., Li, X., Wang, L., Shen, X., et al., (2018). An atypical R2R3 MYB transcription factor increases cold hardiness by CBF-dependent and CBF-independent pathways in apple. *New Phytol.*, 218, 201–218.

- Xiong, H., Li, J., Liu, P., Duan, J., Zhao, Y., Guo, X., Li, Y., et al., (2014). Overexpression of *OsMYB48-1*, a novel MYB-related transcription factor, enhances drought and salinity tolerance in rice. *PLoS One*, 9, e92913.
- Xu, D. B., Gao, S. Q., Ma, Y. Z., Xu, Z. S., Zhao, C. P., Tang, Y. M., Li, X. Y., et al., (2014). ABI-like transcription factor gene *TaABL1* from wheat improves multiple abiotic stress tolerances in transgenic plants. *Funct. Integr. Genom.*, 14, 717–730.
- Xu, Z., Ali, Z., Xu, L., He, X., Huang, Y., Yi, J., Shao, H., et al., (2016). The nuclear protein *GmbZIP110* has transcription activation activity and plays important roles in the response to salinity stress in soybean. *Sci. Rep.*, 6, 20366.
- Yadav, N., Taunk, J., Rani, A., Aneja, B., & Yadav, R. C., (2011). Role of transcription factors in abiotic stress tolerance in crop plants. *Clim. Chang. Plant Abiotic Stress Toler.*, 201, 605–640.
- Yamaguchi, K., Takahashi, Y., Berberich, T., Imai, A., Takahashi, T., Michael, A. J., & Kusano, T. A., (2007). A protective role for the polyamine spermine against drought stress in *Arabidopsis*. *Biochem. Biophys. Res. Commun.*, 352, 486–490.
- Yamaguchi-Shinozaki, K., & Shinozaki, K., (2005). Organization of cis-acting regulatory elements in osmotic and cold-stress responsive promoters. *Trends Plant Sci.*, 10, 88–94.
- Yamaguchi-Shinozaki, K., & Shinozaki, K., (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.*, 57, 781–803.
- Yan, H., Jia, H., Chen, X., Hao, L., An, H., & Guo, X., (2014). The cotton WRKY transcription factor *GhWRKY17* functions in drought and salt stress in transgenic *Nicotiana benthamiana* through ABA signaling and the modulation of reactive oxygen species production. *Plant Cell Physiol.*, 55, 2060–2076.
- Yan, L., Liu, Z. Q., Xu, Y. H., Lu, K., Wang, X. F., & Zhang, D. P., (2013). Auto and cross-repression of three *Arabidopsis* WRKY transcription factors *WRKY18*, *WRKY40*, and *WRKY60* negatively involved in ABA signaling. *J. Plant Growth Regul.*, 32, 399–416.
- Yanez, M., Caceres, S., Orellana, S., Bastias, A., Verdugo, I., Ruiz-Lara, S., & Casaretto, J. A., (2009). An abiotic stress-responsive bZIP transcription factor from wild and cultivated tomatoes regulates stress-related genes. *Plant Cell Rep.*, 28, 1497–1507.
- Yang, A., Dai, X., & Zhang, W. H., (2012a). A R2R3-type MYB gene, *OsMYB2*, is involved in salt, cold, and dehydration tolerance in rice. *J. Exp. Bot.*, 63, 2541–2556.
- Yang, G., Zhang, W., Liu, Z., Yi-Maer, A. Y., Zhai, M., & Xu, Z., (2017). Both *JrWRKY2* and *JrWRKY7* of *Juglans regia* mediate responses to abiotic stresses and abscisic acid through formation of homodimers and interaction. *Plant Biol.*, 19, 268–278.
- Yang, L., Tang, R., Zhu, J., Liu, H., Mueller-Roeber, B., Xia, H., & Zhang, H., (2008). Enhancement of stress tolerance in transgenic tobacco plants constitutively expressing *AtIpK2b*, an inositol polyphosphate 6-/3-kinase from *Arabidopsis thaliana*. *Plant Mol. Biol.*, 66, 329–343.
- Yang, S., Vanderbeld, B., Wan, J., & Huang, Y., (2010). Narrowing down the targets: Towards successful genetic engineering of drought-tolerant crops. *Mol. Plant.*, 3, 469–490.
- Yang, S., Xu, K., Chen, S., Li, T., Xia, H., Chen, L., et al., (2019). A stress-responsive bZIP transcription factor *OsbZIP62* improves drought and oxidative tolerance in rice. *BMC Plant Biology*, 19, 1–15.
- Yang, T., Hao, L., Yao, S., Zhao, Y., Lu, W., & Xiao, K., (2016). *TabHLH1*, a bHLH-type transcription factor gene in wheat, improves plant tolerance to pi and n deprivation via

- regulation of nutrient transporter gene transcription and ROS homeostasis. *Plant Physiol. Biochem.*, 104, 99–113.
- Yang, T., Yao, S., Hao, L., Zhao, Y., Lu, W., & Xiao, K., (2016). Wheat bHLH-type transcription factor gene *TabHLH1* is crucial in mediating osmotic stresses tolerance through modulating largely the ABA-associated pathway. *Plant Cell Rep.*, 35, 2309–2323.
- Yang, X., He, K., Chi, X., Chai, G., Wang, Y., Jia, C., Zhang, H., et al., (2018). Miscanthus NAC transcription factor *MINAC12* positively mediates abiotic stress tolerance in transgenic *Arabidopsis*. *Plant Sci.*, 277, 229–241.
- Yang, X., Kim, M. Y., Ha, J., & Lee, S. H., (2019). Over-expression of the soybean NAC gene *GmNAC109* increases lateral root formation and abiotic stress tolerance in transgenic *Arabidopsis* plants. *Front Plant Sci.*, 10, 1036.
- Yang, Y., Al-Baidhani, H. H. J., Harris, J., Riboni, M., Li, Y., Mazonka, I., Bazanova, N., Chirkova, L., Hussain, S. S., Hrmova, M., et al., (2019). DREB/CBF expression in wheat and barley using the stress-inducible promoters of HD-Zip I genes: Impact on plant development, stress tolerance and yield. *Plant Biotechnol. J.*, 18, 829–844.
- Yang, Z., Chi, X., Guo, F., Jin, X., Luo, H., Hawar, A., Chen, Y., et al., (2020). *SbWRKY30* enhances the drought tolerance of plants and regulates a drought stress-responsive gene, *SbRD19*, in sorghum. *J. Plant Physiol.*, 246, 153142.
- Yao, P. F., Li, C. L., Zhao, X. R., Li, M. F., Zhao, H. X., Guo, J. Y., Cai, Y., et al., (2017). Overexpression of a Tartary buckwheat gene, *FtbHLH3*, enhances drought/oxidative stress tolerance in transgenic *Arabidopsis*. *Front. Plant Sci.*, 8, 625.
- Yao, P., Sun, Z., Li, C., Zhao, X., Li, M., Deng, R., Huang, Y., et al., (2018). Overexpression of *Fagopyrum tataricum* *FtbHLH2* enhances tolerance to cold stress in transgenic *Arabidopsis*. *Plant Physiol. Biochem.*, 125, 85–94.
- Yeo, E. T., Kwon, H. B., Han, S. E., Lee, J. T., Ryu, J. C., & Byun, M. O., (2000). Genetic engineering of drought resistant potato plants by introduction of the *trehalose-6-phosphate synthase (TPS1)* gene from *Saccharomyces cerevisiae*. *Mol. Cell*, 10, 263–268.
- Ying, S., Zhang, D. F., Fu, J., et al., (2012). Cloning and characterization of a maize bZIP transcription factor, *ZmbZIP72*, confers drought and salt tolerance in transgenic *Arabidopsis*. *Planta*, 235, 253–266.
- Yokotani, N., Sato, Y., Tanabe, S., Chujo, T., Shimizu, T., Okada, K., Yamane, H., Shimono, M., Sugano, S., Takatsuji, H., et al., (2013). *WRKY76* is a rice transcriptional repressor playing opposite roles in blast disease resistance and cold stress tolerance. *J. Exp. Bot.*, 64, 5085–5097.
- Yoon, S., Lee, D. K., Yu, I. J., Kim, Y. S., Choi, Y. D., & Kim, J. K., (2017). Overexpression of the *OsbZIP66* transcription factor enhances drought tolerance of rice plants. *Plant Biotechnol. Rep.*, 11, 53–62.
- Yoshida, Y., Kiyosue, T., Nakashima, K., Yamaguchi-Shinozaki, K., & Shinozaki, K., (1997). Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiol.*, 38, 1095–1102.
- Yu, M., Liu, J., Du, B., Zhang, M., Wang, A., & Zhang, L., (2021). NAC transcription factors *PwNAC11* activated *ERDD1* by interaction with *ABF3* and *DREB2A* to enhance drought tolerance in transgenic *Arabidopsis*. *Intl. J. Mol. Sci.*, 22, 6952.
- Yu, X., Kikuchi, A., Matsunaga, E., Morishita, Y., Nanto, K., Sakurai, N., Suzuki, H., et al., (2009). Establishment of the evaluation system of salt tolerance on transgenic woody plants in the special netted-house. *Plant Biotechnol.*, 26, 135–141.

- Yu, Y. T., Wu, Z., Lu, K., Bi, C., Liang, S., Wang, X. F., & Zhang, D. P., (2016). Overexpression of the *MYB37* transcription factor enhances abscisic acid sensitivity and improves both drought tolerance and seed productivity in *Arabidopsis thaliana*. *Plant. Mol. Biol.*, 90, 267–279.
- Yu, Y., Ni, Z., Chen, Q., & Qu, Y., (2017). The wheat salinity-induced R2R3-MYB transcription factor *TaSIM* confers salt stress tolerance in *Arabidopsis thaliana*. *Biochem. Biophys. Res. Commun.*, 491, 642–648.
- Yu, Y., Wang, L., Chen, J., Liu, Z., Park, C. M., & Xiang, F., (2018). *WRKY71* acts antagonistically against salt-delayed flowering in *Arabidopsis thaliana*. *Plant Cell Physiol.*, 59, 414–422.
- Yuan, X., Wang, H., Cai, J., Bi, Y., Li, D., & Song, F., (2019). Rice NAC transcription factor *ONAC066* functions as a positive regulator of drought and oxidative stress response. *BMC Plant Biol.*, 19, 278.
- Zandalinas, S. I., Sales, C., Beltran, J., Gómez-Cadenas, A., & Arbona, V., (2017). Activation of secondary metabolism in citrus plants is associated to sensitivity to combined drought and high temperatures. *Front. Plant Sci.*, 7, 1954.
- Zhai, Y., Zhang, L., Xia, C., Fu, S., Zhao, G., Jia, J., & Kong, X., (2016). The wheat transcription factor, *TabHLH39*, improves tolerance to multiple abiotic stressors in transgenic plants. *Biochem. Biophys. Res. Commun.*, 473, 1321–1327.
- Zhang, C., Liu, J., Zhao, T., Gomez, A., Li, C., Yu, C., Li, H., Lin, J., Yang, Y., Liu, B., et al., (2016). A drought-inducible transcription factor delays reproductive timing in rice. *Plant Physiol.*, 171, 334–343.
- Zhang, J. Y., Broeckling, C. D., Blancaflor, E. B., Sledge, M. K., Sumner, L. W., & Wang, Z. Y., (2005). Overexpression of WXP1, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). *Plant J.*, 42, 689–707.
- Zhang, J. Y., Broeckling, C. D., Sumner, L. W., & Wang, Z. Y., (2007). Heterologous expression of two *Medicago truncatula* putative ERF transcription factor genes, WXP1 and WXP2, in *Arabidopsis* led to increased leaf wax accumulation and improved drought tolerance, but differential response in freezing tolerance. *Plant Mol. Biol.*, 64, 265–278.
- Zhang, L., Zhang, L., Xia, C., Zhao, G., Jia, J., & Kong, X., (2016). The novel wheat transcription factor *TaNAC47* enhances multiple abiotic stress tolerances in transgenic plants. *Front. Plant Sci.*, 6, 1174.
- Zhang, L., Zhang, L., Xia, C., Zhao, G., Liu, J., Jia, J., et al., (2015). A novel wheat bZIP transcription factor, *TabZIP60*, confers multiple abiotic stress tolerances in transgenic *Arabidopsis*. *Physiol. Plant.*, 153, 538–554.
- Zhang, P., Wang, R., Yang, X., Ju, Q., Li, W., Lu, S., Tran, L. P., & Xu, J., (2020). The R2R3-MYB transcription factor *AtMYB49* modulates salt tolerance in *Arabidopsis* by modulating the cuticle formation and antioxidant defense. *Plant Cell Environ.*, 43, 1925–1943.
- Zhang, Q., Wang, M., Hu, J., Wang, W., Fu, X., & Liu, J. H., (2015). *PtaABF* of *Poncirus trifoliata* functions in dehydration tolerance by reducing stomatal density and maintaining reactive oxygen species homeostasis. *J. Exp. Bot.*, 66, 5911–5927.
- Zhang, T., Mo, J., Zhou, K., Chang, Y., & Liu, Z., (2018). Overexpression of *Brassica campestris* *BcICE1* gene increases abiotic stress tolerance in tobacco. *Plant Physiol. Biochem.*, 132, 515–523.

- Zhao, J. S., Ren, W., Zhi, D. Y., Wang, L., & Xia, G. M., (2007). *Arabidopsis DREB1A/CBF3* bestowed transgenic tall fescue increased tolerance to drought stress. *Plant Cell Rep.*, 26, 1521–1528.
- Zhao, K., Zhang, D., Lv, K., Zhang, X., Cheng, Z., Li, R., Zhou, B., & Jiang, T., (2019). Functional characterization of poplar *WRKY75* in salt and osmotic tolerance. *Plant Sci.*, 289, 110259.
- Zheng, P., Allen, W. B., Roesler, K., Williams, M. E., Zhang, S., Li, J., et al., (2008). A phenylalanine in DGAT is a key determinant of oil content and composition in maize. *Nat. Genet.*, 40, 367–372.
- Zheng, X., Chen, B., Lu, G., & Han, B., (2009). Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. *Biochem. Biophys. Res. Commun.*, 379, 985–989.
- Zheng, Y., Ge, J., Bao, C., Chang, W., Liu, J., Shao, J., Liu, X., et al., (2020). Histone deacetylase *HDA9* and *WRKY53* transcription factor are mutual antagonists in regulation of plant stress response. *Mol. Plant.*, 13, 598–611.
- Zhong, L., Chen, D., Min, D., Li, W., Xu, Z., Zhou, Y., Li, L., et al., (2015). *AtTG44*, a bZIP transcription factor, confers drought resistance by enhancing nitrate transport and assimilation in *Arabidopsis thaliana*. *Biochem. Biophys. Res. Commun.*, 457, 433–439.
- Zhou, Q. Y., Tian, A. G., Zou, H. F., Xie, Z. M., Lei, G., Huang, J., Wang, C. M., et al., (2008). Soybean WRKY-type transcription factor genes, *GmWRKY13*, *GmWRKY21*, and *GmWRKY54*, confer differential tolerance to abiotic stresses in transgenic *Arabidopsis* plants. *Plant Biotechnol. J.*, 6, 486–503.
- Zhu, J. K., (2016). Abiotic stress signaling and responses in plants. *Cell*, 167, 313–324.
- Zhu, M., Chen, G., Zhang, J., Zhang, Y., Xie, Q., Zhao, Z., Pan, Y., & Hu, Z., (2014). The abiotic stress-responsive NAC-type transcription factor *SINAC4* regulates salt and drought tolerance and stress-related genes in tomato (*Solanum lycopersicum*). *Plant Cell Rep.*, 33, 1851–1863.
- Zhu, M., Meng, X., Cai, J., Li, G., Dong, T., & Li, Z., (2018). Basic leucine zipper transcription factor *SlbZIP1* mediates salts and drought stress tolerance in tomato. *BMC Plant Biol.*, 18, 1–14.
- Zhu, N., Cheng, S., Liu, X., Du, H., Dai, M., Zhou, D. X., Yang, W., & Zhao, Y., (2015). The R2R3-type MYB gene *OsMYB91* has a function in coordinating plant growth and salt stress tolerance in rice. *Plant Sci.*, 236, 146–156.
- Zhu, S., Shi, W., Jie, Y., Zhou, Q., & Song, C., (2020). A MYB transcription factor, *BnMYB2*, cloned from ramie (*Boehmeria nivea*) is involved in cadmium tolerance and accumulation. *PLoS One*, 15, e0233375.
- Zwack, P. J., & Rashotte, A. M., (2015). Interactions between cytokinin signaling and abiotic stress responses. *J. Exp. Bot.*, 66, 4863–4871.



# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

## CHAPTER 8

---

# Genetics and Microarray in Environmental Stress Response

ROBAB SALAMI,<sup>1</sup> MASOUMEH KORDI,<sup>1</sup> NASSER DELANGIZ,<sup>2</sup>  
BEHNAM ASGARI LAJAYER,<sup>3\*</sup> and TESS ASTATKIE<sup>4</sup>

*<sup>1</sup>Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran*

*<sup>2</sup>Department of Plant Biotechnology and Breeding, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

*<sup>3</sup>Health and Environment Research Center, Tabriz University of Medical Science, Tabriz, Iran*

*<sup>4</sup>Faculty of Agriculture, Dalhousie University, Truro, NS B2N 5E3, Canada, E-mail: astatkie@dal.ca*

*\*Corresponding author. E-mail: asgarihemayat@gmail.com*

---

### ABSTRACT

Abiotic stresses reduce crop yield by about 50% worldwide. Analysis of plants under abiotic stresses can identify the hub genes, which can help to coordinate the plant's response to these stresses and help to detect signaling events to determine plant stress tolerance in the natural environment. The difference in the response of plants to stress is related to regulatory genes. Regulatory genes are genes whose products have a great effect, such as hormones, enzymes, TFs, etc. In the microbial technique, thousands of genes can be expressed simultaneously in the shortest possible time and the stressed plant can be compared to the control plant. In recent years, this technique has produced a large amount of gene expression data. Identifying genes that can



have multiple responses to environmental stresses is very important for the resilience of crops to environmental distresses.

## **8.1 INTRODUCTION**

Environmental stress are some of the factors limiting the growth and yield of crops and plays an important role in the ecological distribution of plants. The most important environmental stresses are heat, cold, salinity, and drought. To deal with these stresses, plants regulate the expression of various genes in the nucleus, cytoplasm, chloroplasts, and other organelles (Zhang et al., 2020).

In plant adaptation to stress, several mechanisms are activated at the molecular, biochemical, and physiological levels that contribute to yield stability. The ability to respond to environmental stresses in different types of plants, and even different plant species is different and needs further investigation. Studies have shown that several systems of regulation of gene expression are involved in the response to stress (Shinozaki & Yamaguchi-Shinozaki, 1997). Accurate knowledge of tolerance mechanisms and genes involved in stress response can improve tolerance to various stresses in crops by methods such as genetic engineering. Therefore, identifying and understanding different pathways of response to stresses has a very important role in improving plant performance (Lee et al., 2005).

Transcriptomics refers to RNA copies of a particular type of cell or tissue under specific developmental, physiological, and environmental conditions and includes rRNA, tRNA, mRNA, and non-coding RNA. The transcriptome of each cell is the expression pattern of actively expressed genes at a particular time; mRNA degradation also affects the transcriptome. Transcriptomics focuses on the expression of genes by examining RNA levels (Taft et al., 2010).

Microarray technology is one of the first tools for transcriptome analysis in the last two decades, which can study the expression levels of thousands of genes simultaneously and provides a profile of the gene expression under stress. In addition, DNA microarray today provides the basis for next generation sequencing (NGS) (Wöhrle et al., 2020) and the models in how the research, techniques, and knowledge in life sciences and biotechnology (including genomics, proteomics, and bioinformatics) would be conducted, presented, and used (Fajriyah, 2021). Identification and selection of responsive genes that increase resistance to environmental stresses in plants is very important. Using techniques such as DNA microarrays, hub genes can be introduced and then used to improve plant resistance to stress. The different aspects of using the microarray technique are explained in this chapter.

## 8.2 ENVIRONMENTAL STRESSES AND METHODS FOR STUDYING THE GENES THAT RESPOND TO THEM

Environmental stresses are the most important factors that reduce the performance of agricultural products worldwide. Environmental stresses include temperature, drought, salinity, flooding, quantity, and quality of light, atmospheric CO<sub>2</sub> concentration, soil nutrients, and heavy metal content (Chen & Soltis, 2020). Resistance to environmental stresses is a quantitative trait that controls many genes. To understand this trait, the gene network, which includes various genes that are involved in controlling the desired traits must be examined. The genes in a gene network definitely have common cis-elements (CRM) and can have different cis-elements. Different datasets are used to draw the gene network and high throughput methods are used to evaluate the gene network.

There are different methods of determining the quantity and quality of transcripts, which fall into three general categories:

- Hybridization-based approaches:
  - Northern blot;
  - Microarray (Table 8.1).
- PCR-based approaches:
  - Quantitative PCR (qPCR);
  - Real-time PCR.
- Sequencing-based approaches:
  - Sanger;
  - SAGE;
  - MPSS;
  - RNA-Seq.

High throughput methods for studying gene expression in plants are SAGE, DD PCR (differential display PCR), microarray and RNA-Seq. In these methods, transcripts of many genes are examined simultaneously.

**TABLE 8.1** Some of the Purposes of Performing Microarray

Types of Chips	Target	Confirmation by
cDNA chip	Investigation of gene expression	Northern blot and real time PCR
DNA chip	Find SNP in DNA sequence	Southern blot
Protein chip	Testing a large number of proteins	Western blot

Microarray technology has been used successfully to simultaneously study the expression of thousands of genes in a wide range of genomics, such as resistance gene identification, drug discovery, and clinical diagnoses (Beltrame et al., 2007). Gene expression level data contain valuable information about biological networks, cell states, and understanding of gene function. One purpose of analyzing gene expression data is to determine how the expression of each individual gene affects the expression of other genes in the same genetic network. Another goal is to determine how genes are expressed in the control and in the treatment groups.

### 8.3 MICROARRAY TECHNOLOGY TO STUDY ENVIRONMENTAL STRESSES

To study environmental stresses microarray technology can be divided into three parts (Figure 8.1):

- **Part I: Chip Design and Preparation:** First, the required information is collected for the genes to be studied, then the nucleotides are put together (by software), and the oligonucleotides are made 17 to 25 bp in length. These probes are placed on a glass slide (Kierzek et al., 2008).

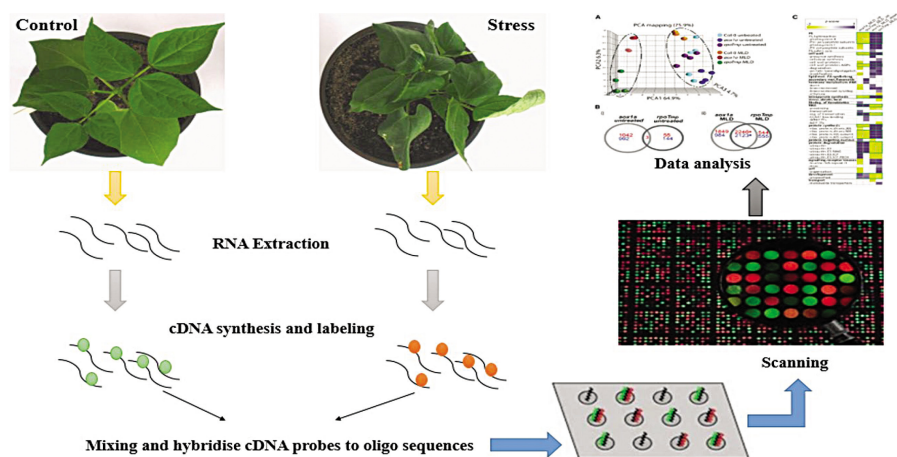


FIGURE 8.1 Microarray technology.

- **Part II: Reaction Preparation:** RNA is first extracted from the target cells or tissues (under normal and stress conditions), and then cDNA is synthesized. Labeling is performed during cDNA synthesis using cy3 and cy5, which are fluorescents of different colors. The cDNAs are then mixed and placed on a microarray chip to hybridize with the oligonucleotide sequence placed on the chip (Southern, 1975).
- **Part III: Analysis of Data:** After hybridization, elution is performed to wash labeled cDNAs that have not hybridized to a 25-nucleotide sequence. Then bioinformatics scans and examinations are performed (Southern, 1975).

#### 8.4 LIMITATIONS OF MICROARRAY TECHNOLOGY

Controlling variability: DNA microarray is only for testing known genes. For genes with low expression levels, the results of microarrays are significantly different. To ensure that the obtained results are reproducible, and to reduce the number of false positive results, it is always better to repeat. Sometimes the total RNA is low and insufficient, and the amplification step needs to be performed, which may cause bias (Chua et al., 2003).

In DNA microarray, variation hybridization occurs, meaning that a piece of the well is hybridized elsewhere. It is difficult to normalize the results in microarray technology; for example, the image quality obtained from wells may be inadequate and the colors produced may be difficult (Varallyay et al., 2008). Data retrieval from databases is difficult and its analysis requires expertise.

#### 8.5 EXAMPLES OF IDENTIFYING GENES IN RESPONSE TO ABIOTIC STRESSES USING MICROARRAYS

A study has been conducted to investigate the effects of different combinations of environmental stresses on the transcript level of the Arabidopsis genome using microarray databases. Plants often respond to water deficiency at the molecular and cellular levels, and a set of genes with different patterns are induced or silenced (Shinozaki & Yamaguchi-Shinozaki, 2007).

Research has shown that the processes that lead to increased stress tolerance and ultimately plant adaptation are regulated by signaling pathways. The result of activating these pathways is regulating the expression

of stress-related genes, which can include activating or deactivating them. Reconstruction of the involved gene network and identification of key genes involved in drought stress in barley were performed using microarray data analysis. Finally, genes involved in response to drought stress were identified based on analysis of related microarray experimental data, protein interaction networks were reconstructed, and Hub genes were identified, including transcription factors and HYH and HY5, DREB2A, DREB1A, ABF3, ARF1, ABI1, and PP2CA phosphatase proteins (Javadi et al., 2017).

Drought stress tolerance, as a complex trait, includes a set of changes including physiological, morphological, and biochemical changes at the plant level (Zhang, 2007). Extensive studies have been performed on all plant species to identify drought stress response genes (Tran et al., 2010). In rice, drought-responsive genes were identified using expression profiles such as microarrays, Expression Sequence Tags (ESTs), RNA gel blot, and qRT-PCR (Degenkolbe et al., 2009). However, a collection of genes responding to drought stress, especially those genes that have significant expression changes compared to normal conditions, is essential to the performance of the set of these genes in drought tolerance (Hruz et al., 2008).

In drought stress, ABA reduces the concentration of potassium in the protective cells of the stomata, leading to the closure of the stomata, which reduces water loss from the guard cells. The expression of many genes induced by drought stress has been identified through microarray whose products increase stress tolerance and regulate the expression of other genes through signaling pathways. Most of these genes are associated with increased levels of ABA in plants (Raghavendra et al., 2010). The ABI3/VP1 protein family has been reported to help ABA to increase grain filling and stress resistance (Kermode, 2005). Application and analysis of microarray results in *Arabidopsis* mutants showed that 73% of genes were affected by VP1 and ABA, indicating a close relationship between ABA signaling and VP1 function (Suzuki et al., 2003).

Under non-stress conditions, Reactive oxygen species (ROS) are present in small and controlled amounts in plant cells (Dumont & Rivoal, 2019). Stress increases the production of ROS, which are toxic and cause severe damage to cellular components. In order to inhibit and collect oxygen free radicals, plants produce various enzymatic and non-enzymatic antioxidants in their cells (Sharm et al., 2012). Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) (Alscher et al., 2002). Using microarray

data, in salinity, cold, heat, and light stresses, the regulation of six antioxidant enzymes in 10 natural *Arabidopsis* ecotypes was evaluated. The expression profiles of 36 genes encoding six enzymatic antioxidants including CSD1-3, FSD1-3, MSD1-2, CAT1-3, APX1-6, APXT, APXS, GPX1-8, MDAR1-5, and DHAR1-4 were evaluated. In particular, the FSD1, FSD2, CSD1, and CSD2 genes encode SOD. CAT2 and CAT3 for CAT; APX3-6, APXT, and APXS for APX, GPX1, GPX2, GPX5, GPX6, and GPX7 for GPX. In low light and cold stresses, it further up-regulates the expression of genes (Filiz et al., 2019).

Salinity is one of the most important environmental stresses that reduces growth and yield in plants (Hill et al., 2016). The expression pattern of 1,728 genes in response to salinity stress in rice was investigated by microarray method (Kamaluldeen et al., 2014). Also, a study examined the expression pattern of 32,000 genes in response to salinity stress in wheat, a total of 3,416 genes increased in salinity and 2,580 genes showed decreased expression (Kawaura et al., 2008). As previously mentioned, low throughput methods are used to confirm the results of microarrays, for example, in a study by Salami et al. (2017), the expression analysis of Hv TIP2;3 and Hv TIP4;1 in barley genotypes under salinity stress was performed by real-time method. Identification of new genes and determination of their expression pattern is done with the aim of adapting plants to different types of stresses, and in this regard, effective solutions should be created in plant modification to improve stress tolerance.

## 8.6 CONCLUSION

Plants are subject to various environmental stresses during their growth and development. The response to these stresses involves changes at the cellular and molecular levels. Microarray provides a new opportunity for biological search, including the explanation of genes in specific processes such as cell cycle, growth, and development, the evaluation of the effects of chemical and genetic disorders, and the identification of genes. Identification of tolerance mechanisms and genes involved in stress response with the help of powerful microarray technology can help to take measures to improve tolerance to various stresses in crops and improve their performance by methods such as genetic engineering. Microarray is based on gene network. Different methods can be proposed to reconstruct gene regulatory networks, such as protein interaction networks and gene expression networks to identify hub genes.

## KEYWORDS

- **abiotic stresses**
- **crop adaptation**
- **expression sequence tags**
- **glutathione peroxidase**
- **reactive oxygen species**
- **regulatory genes**
- **superoxide dismutase**

## REFERENCES

- Alscher, R. G., Erturk, N., & Heath, L. S., (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.*, 53, 1331–1341.
- Beltrame, F., Papadimitropoulos, A., Porro, I., Scaglione, S., Schenone, A., & Tortorolo, L., (2007). GEMMA-A Grid environment for microarray management and analysis in bone marrow stem cells experiments. *Fut. Generat. Computer Syst.*, 23(3), 382–390.
- Chen, Z. H., & Soltis, D. E., (2020). Evolution of environmental stress responses in plants. *Plant Cell Environ.*, 43(12), 2827–2831.
- Chua, M. Z., Mansfield, E., & Sarwal, M., (2003). Applications of microarrays to renal transplantation: Progress and possibilities. *Front. Biosci.*, 8, s913–923.
- Degenkolbe, T., Do, P. T., Zuther, E., Repsilber, D., Walther, D., Hinch, D. K., & Köhl, K. I., (2009). Expression profiling of rice cultivars differing in their tolerance to long-term drought stress. *Plant Mole. Biol.*, 69(1, 2), 133–153.
- Dumont, S., & Rivoal, J., (2019). Consequences of oxidative stress on plant glycolytic and respiratory metabolism. *Front. Plant Sci.*, 10, 166.
- Fajriyah, R., (2021). An overview on microarray technologies. *Bull. Appl. Math. Mathematics Educ.*, 1(1), 21–30.
- Filiz, E., Ozyigit, I., Saracoglu, I. A., Uras, M. E., Sen, U., & Yalcin, B., (2019). Abiotic stress-induced regulation of antioxidant genes in different *Arabidopsis* ecotypes: Microarray data evaluation. *Biotechnol. Biotechnol. Equip.*, 33(1), 128–143.
- Hill, C. B., Cassin, A., Keeble-Gagnere, G., Doblin, M., & Bacic, A., (2016). De novo transcriptome assembly and analysis of differentially expressed genes of two barley genotypes reveal root-zone-specific responses to salt exposure. *Sci. Rep.*, 6, 31–55.
- Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Widmayer, P., Gruissem, W., & Zimmermann, P., (2008). Genevestigator v3: A reference expression database for the meta-analysis of transcriptomes. *Adv Bioinform.*, 420747. <https://doi.org/10.1111/pce.13922>.

- Javadi, M., Shobbar, Z. S., Ebrahimi, A., & Shahbazi, M., (2017). Reconstruction of gene networks involved in response to drought stress in barley. *New Cell Mol. Biotechnol. J.*, 8(29), 39–48.
- Kamaluldeen, J., Yunusa, I., Zerihun, A., & Bruhl, J., (2014). Uptake and distribution of ions reveal contrasting tolerance mechanisms for soil and water salinity in okra (*Abelmoschus esculentus*) and tomato (*Solanum esculentum*). *Agr. Water Manag.*, 146, 95–104.
- Kawaura, K., Mochida, K., Yamazaki, Y., & Ogihara, Y., (2008). Genome-wide analysis for identification of salt responsive in common wheat. *Func. Integr. Genomics.*, 8, 277–286.
- Kermode, A. R., (2005). Role of abscisic acid in seed dormancy. *J. Plant Growth Regul.*, 24, 319–344.
- Kierzek, E., Barciszewska, M. Z., & Barciszewski, J., (2008). Isoenergetic microarray mapping reveals differences in structure between tRNA<sup>i</sup> met and tRNA<sup>m</sup> met from *Lupinus luteus*. *Nucleic Acids Symp. Ser.*, 52, 215–216.
- Lee, B. H., Henderson, D. A., & Zhu, J. K., (2005). The *Arabidopsis* cold-responsive transcriptome and its regulation by ICE1. *Plant Cell*, 17, 3155–3175.
- Raghavendra, A. S., Gonugunta, V. K., Christmann, A., & Grill, E., (2010). ABA perception and signaling. *Trends Plant Sci.*, 15, 395–401.
- Salami, R., Mohammadi, S. A., Ghaffariyan, S., & Moghaddam, M., (2017). Evaluating the expression of Hv TIP2;3 and Hv TIP4;1 in barley genotypes under different levels of salinity stress. *Indian J. Genetics Plant Breed.*, 77(4), 524–530.
- Sharm, P., Jha, A. B., Dubey, R. S., & Pessarakli, M., (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.*, 217037. doi: 10.1155/2012/217037.
- Shinozaki, K., & Yamaguchi-Shinozaki, K., (1997). Gene expression and signal transduction in water-stress response. *Plant Physiol.*, 115, 327–334.
- Shinozaki, K., & Yamaguchi-Shinozaki, K., (2007). Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.*, 58, 221–227.
- Southern, E. M., (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, 98, 503–511.
- Suzuki, M., Ketterling, M. G., Li, Q. B., & McCarty, D. R., (2003). Viviparous 1 alters global gene expression patterns through regulation of abscisic acid signaling. *Plant Physiol.*, 132(3), 1664–1677.
- Taft, R., Pang, K. C., Mercer, T. R., Dinger, M., & Mattick, J. S., (2010). Non-coding RNAs: Regulators of disease. *J. Pathol.*, 220, 126–139.
- Tran, L. S. P., & Mochida, K., (2010). Functional genomics of soybean for improvement of productivity in adverse conditions. *Func. Integr. Genomics*, 10(4), 447–462.
- Varallyay, E., Burgyan, J., & Havelda, Z., (2008). MicroRNA detection by northern blotting using locked nucleic acid probes. *Nat. Protoc.*, 3, 190–196.
- Wöhrle, J., Krämer, S. D., Meyer, P. A., Rath, Ch., Hügle, M., Urban, G. A., & Roth, G., (2020). Digital DNA microarray generation on glass substrates. *Sci. Rep.*, 10(1), 1–9.
- Zhang, Q., (2007). Strategies for developing green super rice. *Proc. Nat. Acad. Sci.*, 104(42), 16402–16409.
- Zhang, Y., Zhang, A., Li, X., & Lu, C., (2020). The role of chloroplast gene expression in plant responses to environmental stress. *Int. J. Mole. Sci.*, 21(17), 6082. <https://doi.org/10.3390/ijms21176082>.





# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

## CHAPTER 9

---

# Small RNAs – The Big Players in Developing Salt-Resistant Plants

ANUP KUMAR SARKAR<sup>1,2</sup> and SANJOY SADHUKHAN<sup>2\*</sup>

<sup>1</sup>*Department of Botany, Duxhulal Nibaran Chandra College, Aurangabad – 742201, Murshidabad, West Bengal, India*

<sup>2</sup>*Plant Molecular Biology Laboratory, Department of Botany, Raiganj University, Raiganj – 733134, Uttar Dinajpur, West Bengal, India*

*\*Corresponding author. E-mail: sanjoysadhukhan@gmail.com*

---

### ABSTRACT

Salinity is a severe threat to plants which can affect almost all developmental processes of the plant. Salinity is often considered a complex constraint that induces the regulation of many genes with a significant function in the mechanism of salt resistance. Researchers and scientists are working hard to uncover the molecular processes behind salt tolerance in both halophytic and non-halophytic plants. In many studies, it was found that a special category of RNA, often called non-coding small RNA or simply small RNAs (sRNAs) can execute different biological processes in plants against salt-induced stresses. sRNAs are usually 20–30 nucleotides long and directly participated in the regulation of almost 30% or more of the genes in a cell and are broadly categorized into two broad groups microRNA (miRNA) and short interfering RNAs (siRNAs). Gradual scientific efforts have reported the significance of different subcategories of sRNAs including microRNAs (miRNAs), short interfering RNAs (siRNAs), natural antisense transcript derived siRNAs (nat-siRNA), trans-acting siRNA (ta-siRNA), etc., in salinity stress response in the plant. To date, many halophytic and non-halophytic plants have been

studied to determine the contribution of various sRNAs in salt tolerance, as well as the molecular basis of salt stress regulation in plants. Advancement of computational Biology and innovation of various tools of Bioinformatics also accelerates the genome-wide identification of these sRNAs, as well as their targets. Various researchers are trying to use the sRNAs mediated RNA interference approach for the development of resistant varieties of various crops for combating soil salinity as well as other abiotic stresses. In this chapter, the authors have presented the fundamental and applied aspects of small RNA and RNA interference technology in developing salt-resistant plants.

## **9.1 INTRODUCTION**

Organisms have evolved with a gamut of diverse and sophisticated mechanisms for survival and growth during stressful situations caused by several biotic and abiotic environmental factors. Plants are primarily sessile organisms, and so they set up multiple layers of protective measures to respond and resist the surrounding adverse environment (Zhang & Hong, 2019). The stress response of plants to abiotic and biotic variables has been addressed in recent decades by employing certain traditional as well as contemporary breeding methods, which has resulted in crop improvement to some extent. Extensive understanding of adaptive reactions in the plant to stressful situations will expedite the development of novel approaches for improving plant tolerance against stresses (Kumar, 2014). It is well accepted that most of the plants activate reprogramming of gene expression through regulation at the transcriptional level or translational level to stimulate defensive mechanisms for survival under abiotic stress conditions. Among these abiotic variables, soil salinity is regarded as one of the most detrimental due to its proclivity to impose constraints on the majority of plants. Accumulation of salt in the soil has resulted from some natural and anthropogenic activities that limit the global productivity of crops, as it has been projected that 20% of global cultivable land is highly affected by salts (Younis et al., 2010). Soil salinity has a negative influence on growth and development, which can lead to abnormalities or even early death. There is no doubt that the salt tolerating cultivars of crop plants have high demand in international markets, as the majority of the arable land worldwide is gradually getting saline and dry. To overcome such conditions different research activities are being carried out on various crop plants by using biotechnological approaches for pointing out and characterization of different genes under salt stress as well

as salt-induced drought stress responses. Technological advancement has also enlightened the influence of salinity stress on structural conformations, function features and networks of the transcription factors and it is crucial to identify the particular role of each transcription factor or gene prior to gene modification during the creation of salt-resistant cultivar. Most of the eukaryotic organisms adopted some small RNA-based mechanisms to withstand unfavorable conditions (Agrawal et al., 2003). Creditable research on various plants has identified the function of some small RNAs (sRNAs), which significantly regulate salt tolerance responses. To date, an enormous number of sRNAs together with their biogenesis and mechanism of action have been recognized in many crop plants. Endogenous sRNAs, which are linked with the genetic control of many morphological and physiological traits in plants, are increasingly regarded as essential factors in the regulation of salt tolerance in plants. Gene regulation is generally executed through the condensation and transcriptional repression of chromatin, i.e., formation of heterochromatin and it is essential for controlling gene expression in every event of growth and development including silencing parasitic gene elements, genome stability and inheritance (Johnson & Straight, 2017). Significant research has established that RNA interference (RNAi) and heterochromatin production are linked, but they were previously considered to be regulated by entirely distinct, even unrelated processes (Martienssen & Moazed, 2015). RNA interference (RNAi) is now specifically used to refer the gene silencing, which is executed by exogenous double-stranded RNA (dsRNA), but in a broader sense, it refers the all the gene silencing, which is triggered by sRNAs in combination with a representative of the Argonaute protein family (Fire et al., 1998; Martienssen & Moazed, 2015). Comprehensive knowledge about sRNA mediated molecular mechanisms of stress responses would provide a thorough understanding of the initiation and progression of stress tolerance and is essential to develop stress-resistant plants. Adequate understanding over the last couple of decades has indicated that sRNAs are more common than previously assumed. Ongoing and forthcoming investigations will optimistically provide more needful and detailed to combat effectively salt-induced stress as well as other kinds of abiotic stresses.

## 9.2 SALINITY

Salinity is regarded as a decisive abiotic stress factor that is deleterious for most crop plants. Globally, it causes enormous losses to crop production, and

since few decades it has gained the focus of extensive research to unlock the complicated mechanisms entailed in salt tolerance in plants. In comparison to other abiotic stresses, salinity is quite complex as it exerts both osmotic and ionic action that accelerates its toxicity greatly (Parmar et al., 2020). Plants also develop various adaptive measures to exclude excess salt from the cell and also develop some modifications to tolerate salt within their cells for survival under salinity (Munns & Tester, 2008). Different plant species show different adaptive features under salinity and their responses also vary to salinity. In most cases, salt-sensitive plants experience osmotic stress, ionic toxicity, and oxidative stress and in severe cases, salinity causes the death of the plant; on the contrary, to cope with salinity, salt-tolerant plants employ a variety of morphological, physiological, and molecular processes (Bartels & Sunkar, 2005; Munns & Tester, 2005; Zhang & Shi, 2013). Excess salinity inhibits water uptake by the root system and reduces turgor pressure water efflux from the vacuole, thereby causing insufficient osmotic imbalance (Devkar et al., 2020). Any salt-sensitive plant growing under salinity suffers from triple-fold impacts, viz. osmotic stress, ionic stress, and oxidative stress and thus become incapable to grow further (Khare et al., 2015; Surekha et al., 2015; Wani & Gosal, 2010). There is a real need for some effective strategies for developing salt-tolerant cultivars and salt remediation. Most of the crop plants that are salt sensitive are called glycophytes. To defend and survive during salt-induced stress conditions glycophytic plants have developed elaborate and systematic sensory and adaptive responses including gene transcription network and activation of signaling cascade (Joshi et al., 2016; Kinoshita & Seki, 2014; Wani et al., 2013). Plant stress tolerance usually associated with morphological, physio-chemical, and molecular mechanisms, which are ultimately regulated by genes. Based on the model plant *Arabidopsis* researchers have identified many genes which are required for salt tolerance and a few of them are successfully used to increase salt tolerance levels in some agriculturally important crop cultivars through genetic engineering and biotechnological approaches (Zhao et al., 2020). Plant breeders of every corner of the world seek to develop and identify cultivars that are more tolerant towards salinity. Many genes are critically involved in several kinds of plant responses against salt-induced stress. Some of the genes are associated with the activation of ion channels, whereas others are count in signal transduction and modification of growth-regulating factors for plant architecture, chiefly root morphology (Mirlohi & He, 2016). Every response of plants to salt stress is the result of a complex and dynamic mechanism governed by many gene loci and carried

out by transcriptional machinery in collaboration with a complex system of transcription factors (Agarwal et al., 2013). Alarming data have shown salt stress potentially regulates numerous plant genes both at transcriptional and post-transcriptional levels in different species, underscoring the role of usual transcriptional regulators during stress (Deinlein et al., 2014; Golldack et al., 2011). Manipulation of such transcription factors and transcription system plants showed many adaptive features such as overexpression of the stress-responsive gene, activity of several transporters, biogenesis of various protective metabolic substances such as osmolytes, polyamines, antioxidative enzymes, etc. (Hasegawa, 2013; Yadav et al., 2012). Different kinds of post-transcriptional and post-translational modifications such as sumoylation, ubiquitination, etc., also perform a very important role in the control of gene expression during stressful situations and collectively such strategies help plants to achieve adaptive modification as well as capability of survival under salinity (Chen & Aravin, 2015).

### 9.3 SMALL RNA AND ITS TYPIIFICATION

Over a long period, RNA was thought to be a transitional component that serves as a bridge between gene loci in DNA and polypeptides (mRNAs) or to be a molecule which is serving effective roles during gene splicing (snRNA) or to be the components of protein-synthesizing machinery (t-RNA and rRNA). The subsequent invention of many non-coding small RNA molecules with distinct regulatory roles has modified the overall concept regarding gene regulations and gene expressions. sRNAs are 18–30 nucleotides in length and have a large effect on several metabolic regulations including plant defense responses. Categorization of small RNAs is quite difficult because of their biogenesis, polarity, and extraordinary differences in transcript length and based on these criteria sRNA is generally subcategorized into two distinct groups: miRNA or microRNA and siRNA or short interfering RNAs; however, they are often subcategorized into several other forms. Biochemical and functional properties of both subcategories are nearly identical, and both of these are 19–20 nt long with 5'-phosphate and 3'-hydroxyl end and in both cases mode of action to silent gene expression is involved RNA-induced silencing complex (RISC) (Ambros et al., 2003; Kim, 2005a). After the discovery of plant small RNA (sRNA) in *Arabidopsis* in 2002, molecular biologists of various corners of the globe engaged to invent small RNAs from the various organism and their cumulative effort explored not only the numerous sRNAs but also determined their mode of

regulation on several biological processes including cellular metabolisms, seed germination, growth, and development events, adaptive responses against abiotic stresses (Chen et al., 2018; Llave et al., 2002). The majority of plant sRNA comes from one of these three pathways: miRNA biogenesis, secondary siRNA biogenesis, or heterochromatic siRNA (hc-siRNA) biogenesis (Borges & Martienssen, 2015). sRNA biogenesis may be regarded as a mechanism that enables plants to reorganize gene expression pathways needed for adjustment and survival under stressful conditions (Phillips et al., 2007). Major characteristics of small RNAs (s-RNAs) are:

1. sRNAs are riboregulators that have pivotal roles in the majority of the biological processes of eukaryotic organisms. They control gene expression in several ways. In some cases, sRNAs act on DNA and execute sequence elimination and chromatin remodeling, whereas in some other cases, sRNAs act on RNA molecules and execute cleavage and translation repression.
2. Usually, sRNAs are made from dsRNA few recent investigations enlightened some new RNA-silencing pathways wherein sRNAs are generated from long-stranded RNA (ssRNA) precursors (Martienssen & Moazed, 2015).
3. In the context of plant science, sRNAs have an unusual convolution, and they are classified according to their biogenesis and the conformation of genome loci from which these are generated. To date, sRNAs are classified into two broad categories microRNAs (miRNAs) and interfering RNAs (siRNAs). As per the knowledge of the latest literature in this field, siRNAs are further classified into three subgroups, namely natural antisense siRNA (natsiRNA), trans-acting siRNA (ta-siRNA) and hc-siRNA. As a result of the steady analysis of large-scale siRNA sequencing efforts, the number of siRNA subgroups may expand in the near future (Phillips et al., 2007).
4. Until now sRNAs in plants have been found to be between 20 nt and 24 nt long (Hackenberg et al., 2015).
5. All variants of characterized sRNA in plants associate with a special class of endonucleases, known as – ARGONAUTE (AGO) family proteins and collectively these two form a specialized ribonucleoprotein complex-RNA induced silencing complexes (RISCs). sRNAs guide the complex to bind with DNA through base pairing or with target RNA (Peters & Meister, 2007; Vaucheret, 2008).

6. For all categories of sRNA, biogenesis takes place through four basic steps.: (i) Transcription of sRNA is brought about by dsRNA and an RNA polymerase (RNAP) act as a driving agent of the transcription of the corresponding gene; (ii) then the dsRNA has become 18–25 nucleotide long sRNA; (iii) next to this 3' end of sRNA become methylated; and (iv) ultimately the sRNA binds with effector complex, i.e., RISC, which allow it to detect and interact with its target site (Voinnet, 2009; Budak & Akpinar, 2015).
7. Generally, RNase III type ribonuclease Dicer-like (DCL) protein control the processing of sRNA and after incorporation into Argonaute (AGO) proteins, it silences genes at the transcriptional level by modifying DNA or histones, and at the post-transcriptional level either by cleavage and destruction of mRNA or protein repression (Baulcombe, 2004; Chapman & Carrington, 2007).
8. sRNAs play important roles in a wide range of biological processes, along with morphological development, genomic stability, physiological balance, and various biotic and abiotic responses (Vaucheret, 2006). During stress conditions, miRNAs and siRNAs can be upregulated or downregulated, and due to such modifications, the post-transcriptional activity of respective target genes is altered, which in turn results in adaptive responses in plants (Leung & Sharp, 2010).
9. sRNA is a mobile element and can move from a gene locus to another or even from one cell to others and play a significant role in non-cell-autonomous gene silencing, which may be local or systematic in nature (Baldrich et al., 2019).
10. Several researchers confirmed that sRNA can move from plant cells to pathogens and such transferred sRNA permits plants to control the expression of genes in the invading pathogens or parasites through a process called host-induced gene silencing (HIGS) (Baldrich et al., 2019).
11. Although a large number of studies have proven mobile RNAs in the plant, it is still not possible to determine the mechanism by which these RNAs move (Baldrich et al., 2019).
12. Both in the cytoplasmic level and nuclear level sRNA regulate gene expression through versatile and complex mechanisms and it is fact that slight alteration of sRNA mediated regulatory mechanisms may influence normal cellular functions (Catalanotto et al., 2016).



### **9.3.1 MICRORNA (miRNA)**

microRNAs (miRNAs) are single-stranded RNA molecules that are short in length and non-coding. They can bind with the non-coding region in the poly-A tail of mRNA to inhibit the activity or promote the degradation of mRNA (Bartel, 2004). These are abundantly present in the genomes of plants, animals, and viruses, and the vast majority of them have distinct genetic loci and exhibit conservation among species belonging to the same biological kingdom (Lagos-Quintana et al., 2001). The length of miRNAs for animals ranges from 20–22 nucleotides, whereas it is 20–24 nucleotides for plants (Bartel, 2004; Reinhart et al., 2002). miRNAs are reported to regulate gene expression in plants that are involved in a variety of activities, including the development of root, stem, leaf, and flower, shifting of vegetative phase to reproductive phase and stress responses (Zhang et al., 2006). During the biogenesis of miRNAs, the template genes are catalyzed by RNA polymerase II and resulted in a primary transcript that adopts a feedback structure (Yaish et al., 2015). With the assistance of some nuclear proteins, such fold back structure is ultimately converted into a specialized RNA duplex structure often called miR-3P/miR-5P duplex (Kurihara et al., 2006). The complex is then divided to produce mature miRNA. After the formation, mature miRNAs bind with the functional endonuclease protein, i.e., Argonaute (AGO) to form the RISC, which in turn accomplish gene regulation through the splitting of mRNA or cleavage of mRNA or inhibition of translational activities (Naqvi et al., 2012). Both pleiotropically and prevalently, a single miRNA is mostly able to recognize more or less hundreds of distinct mRNA transcripts (Li et al., 2018). To recognize target mRNA such miRNA, use sequence complementarity which is the prerequisite to regulating the level of target protein (Bartel, 2004; Llave et al., 2002; Shukla et al., 2011). The microRNA can recognize multiple mRNAs and can bind with them and in this way mRNA and microRNA jointly consist of a precise regulation system (Zhang & Xie, 2017). Along with normal developmental events and stress response miRNAs are found to be a key player in cell differentiation, proliferation, tumorigenesis, apoptosis, etc., in plants as well as other eukaryotes (Cai et al., 2009). Few miRNAs have been revealed that are highly conserved across distantly related taxa, ranging from mosses to higher flowering eudicots in the plant kingdom and worms to mammals in the animal kingdom (Axtell et al., 2007; Zhang et al., 2006; Bartel, 2004). The binding of miRNA and their target mRNA maybe with perfect

complementary or nearly perfect complementary but in both cases gene regulation is perfectly executed through cleavage, repressed translation or often de-adenylation and in both cases miRNA downregulates the levels of mRNA produced from the target gene and thus, the proteins encoded by the target gene are also reduced (Parmer & Shaw, 2018).

In plants, some miRNAs are highly conserved, whereas some are lineage and species-specific immature miRNA (Sun, 2012). Some of them exist as a single copy and some are as multiple copies or gene clusters in the genome (Si et al., 2014). In the majority of cases, plant miRNA targets transcriptional factors that are involved in all developmental processes, from seed germination to seed production via the formation of a mature plant (Jones-Rhoades et al., 2006). However, miRNAs indirectly regulate such processes by forming RISCs that act on mRNAs produced from target genes, and also control expression through adenylation, translational inhibition, or degradation of target mRNA (Lu et al., 2008; Phillips et al., 2007). Various studies have indicated that stress responses of a miRNA may differ with the variation of the genus or species, while in the case of some miRNAs patterns of stress responses are more or less similar in all plants (Zhang, 2015).

Previously, it was assumed that miRNAs were conserved throughout a wide variety of plant species, including flowering plants and non-flowering plants such as bryophytes and pteridophytes, but later it was proved that few miRNAs are always restricted to some specific genera (Floyd & Bowman, 2004). In plants binding of miRNAs with their target mRNA are based on extensive sequence complementarity, however, there are few exceptions where five or more mismatches have been found between the target mRNA and miRNA (Axtell, 2013). Some researchers have pointed out a few examples to confirm about restricted miRNAs, such as few miRNAs of *Arabidopsis* are not reported in the rice genome, and similarly, miRNAs reported from poplar are absent in *Arabidopsis* (Lu et al., 2005). Allen et al. (2004) conceptualized a model to explain the evolutionary aspect of miRNA genes in plants and this model miRNAs were distinguished into “old miRNA” and “young miRNA” and opined that the former type evolved prior to the origin and evolution of many plant taxa and that is why are conserved, while the latter type originated after the emergence of taxons of the present era and hence are not conserved. This model satisfied the fact that miRNAs present in one plant are not present in other plants (Lu et al., 2005; Sunkar & Zhu, 2004). However, numerous miRNAs have been identified, and their targets are conserved across various plant species (Montes et al., 2014).

### 9.3.2 SHORT INTERFERING RNA

Short interfering RNAs (siRNA) are the small RNAs having a key role in gene silencing (Thakur, 2003). Three kinds of siRNAs with lengths of 21, 22, and 24 nucleotides have been shown to play critical roles in developmental processes and immunity generation in plants (Wu et al., 2020). Initially, the majority of siRNAs are generated as short 3' duplexes by the enzymatic activity of endonucleases from the Dicer family, acting upon long dsRNA (Baulcombe, 2005). Then, through sequence complementarity with target transcripts, one strand of the duplex is loaded into the Argonaute proteins, which collectively regulate the gene expression of the target gene (He et al., 2019). siRNAs assign many proteins which modify DNA and histones, namely cytosine methyltransferase, chromomethylase-3 (Ossowski et al., 2008) and they vigorously modify transcriptionally active chromatin into transcriptionally inactive chromatin state (Kamthan et al., 2015). Endogenous siRNAs are further categorized into several groups such as natural antisense siRNAs (natsiRNAs), miRNA-induced trans-acting siRNAs (tasiRNAs), cis-acting siRNAs (casiRNAs), and hc-siRNAs based on the specific mode of biogenesis and mode of action (Axtell, 2013; Choudhuri, 2009). natsiRNAs are generated by the processing of dsRNAs that were produced from endogenous RNAs having complementary sequences and DCL4 or DCL2 protein acts on the precursors to produce 21- or 22-nucleotide long nat-siRNAs, respectively (Medina et al., 2018). Pha-siRNAs are 21 nt long RNAs that are converted from dsRNA with the help of RDRC and processed with the help of DCL4 (Medina et al., 2008; Vazquez et al., 2004). Another siRNA especially 24 nt long, that are associated with the triggering and modification of *de novo* methylation of desired loci by RNA-directed DNA Methylation (RdDM) method, are called "heterochromatic siRNAs" (hc-siRNA) (Chan et al., 2006; Dalakouras & Wassenegger et al., 1994). In the case of both hc-siRNA and pha-siRNA, Dicer-like proteins (DCL) recognize the precursor RNAs and cleave them into products of defined length (Baldrich et al., 2019). All types of siRNAs are remarkably important for plant gene regulation. siRNAs that have length 21 nt and 24 nt are usually involved in the cleavage of messenger RNAs and DNA methylation (Bologna & Voinnet, 2014; Borges & Martienssen, 2015). Wu et al. (2020) showed that 22 nt long siRNAs that are quite different from 21 nt siRNAs and 24 nt siRNAs, effectively repress translation, triggers transitive RNA interference and amplification of gene silencing but are less effective in target cleavage. Further study concluded that induction and upregulation

of 22 nt siRNAs lead translational slowdown and growth inhibition under certain stress conditions. However, the mechanism of 22 nt siRNAs in the execution of translational repression and transitive RNA interference are still under research that mediate translational repression and transitive RNA interference are still under research.

## **9.4 OTHER TYPES OF SMALL RNA**

In addition to these sRNAs, several classes of small RNAs have been discovered. Some of them are well studied and found to have significant involvement in diverse cellular processes of animals and fungi. Such sRNAs are functionally diverse with scanty information. To date, there is no report about the occurrence of such small RNAs in plant species, but they have pivotal roles in the gene regulation of a few biological phenomena in some members of the animal kingdom (Jones-Rhoades et al., 2006). From a botanical perspective, miRNAs and siRNAs make up an entire portion of the sRNA population.

### **9.4.1 SMALL TEMPORAL RNA (ST-RNA)**

In *C. elegans*, a specialized group of 22 nt long RNAs have been reported that are known as Small Temporal RNA (st-RNA) and are involved in temporal regulation of the developmental process of the organism (Thakur, 2003). Further studies revealed that initially, these are generated from ~ 70-nt ssRNA having a stem-loop structure and after processing, this st-RNA is thought to prevent their target mRNA by binding with the target complementary 3' untranslated regions (Thakur, 2003).

### **9.4.2 PIWI-INTERACTING RNA (PI-RNA)**

Piwi-interacting RNAs (piRNAs) are another type of small RNA that is frequently regarded as a distinct class of miRNAs with a length range between 24 and 32 nucleotides and are mostly expressed in germline cells of some animals (Aravin et al., 2007). These pi-RNAs act as a vital genetic and epigenetic regulatory factor and effectively regulate the stability of germ cells, genome integrity, stability of mRNA, methylation of DNA and control of retrotransposons (Zuo et al., 2016).

## 9.5 BIOGENESIS OF SMALL RNA

The biogenesis of various sRNAs in plants, such as miRNAs and siRNAs, is quite similar. Both of these sRNAs are made from larger RNA precursors by enzymes termed DICER-LIKE, which are ribonuclease-III-like nucleases (DCL). In the context of sRNA biogenesis in animals, the nuclease is known as Dicer. However, there are some basic differences in their processing. Usually, miRNA is produced from the genetic loci called MIR genes (Figure 9.1). Just like other protein-coding genes MIR genes are also first transcribed into a primary transcript (pri-miRNA), which then forms an incomplete “fold-back” stem-loop or hairpin structure due to the existence of intra-molecular sequence complementarity (Guo et al., 2016). The transcription is generally mediated by RNA polymerase II (RNA Pol-II) to yield primary miRNA (pri-miRNA) which is subsequently subjected to 5' capping and 3' polyadenylation (Koroban et al., 2016). In the nucleus, from this pri-miRNA structure, DCL1 produces a pre-miRNA molecule with a specific “stem-loop” structure in collaboration with the dsRNA-binding protein DRB1 or HYPONASTIC LEAVES 1 (HYL1) (Eamens et al., 2009; Kurihara et al., 2006; Kurihara & Watanabe, 2004). According to Voinnet (2009) DCL1, HYL1 and SERRATE proteins have collaboratively executed the processing of pre-miRNA from the primary transcript (pri-miRNA) in plants. The further process of pre-miRNA is mediated by DCL1 within the nucleus, generating a 21 nucleotide long imperfect RNA duplex that is comprised of mature miRNA (guide strand) and newly synthesized miRNA (miRNA rider stand). The RNA methylase HUA ENHANCER1 (HEN1) then methylates the 3' terminal nucleotides of the RNA duplex at the 2'-O-hydroxyl group, which facilitate to shield of the miRNA: miRNA duplex from degradation (Li et al., 2005). After that, an exportin protein HASTY (HST1) release the duplex into the cytoplasm. Inside the cytoplasm, the strand of miRNA duplex which is integrated into the AGO protein regulates enzymatic activity of RISC and directs RISC to connect with related target transcripts through sequence complementarity (Achkar et al., 2016; Kim et al., 2009; Lee et al., 2004).

The siRNA molecules are generated from dsRNAs which may be endogenous or exogenous in origin. Plants generate various kinds of siRNAs viz., trans-acting siRNAs, phased secondary siRNA, natural antisense siRNA, and hc-siRNA. Endogenous sources of siRNAs include dsRNA generated through natural cis-antisense, dsRNA produced from heterochromatin, gene paired encoded mRNAs and also as an outcome of miRNA induced cleavage of ssRNAs (Chinnusamy et al., 2007). Among the exogenous origins of

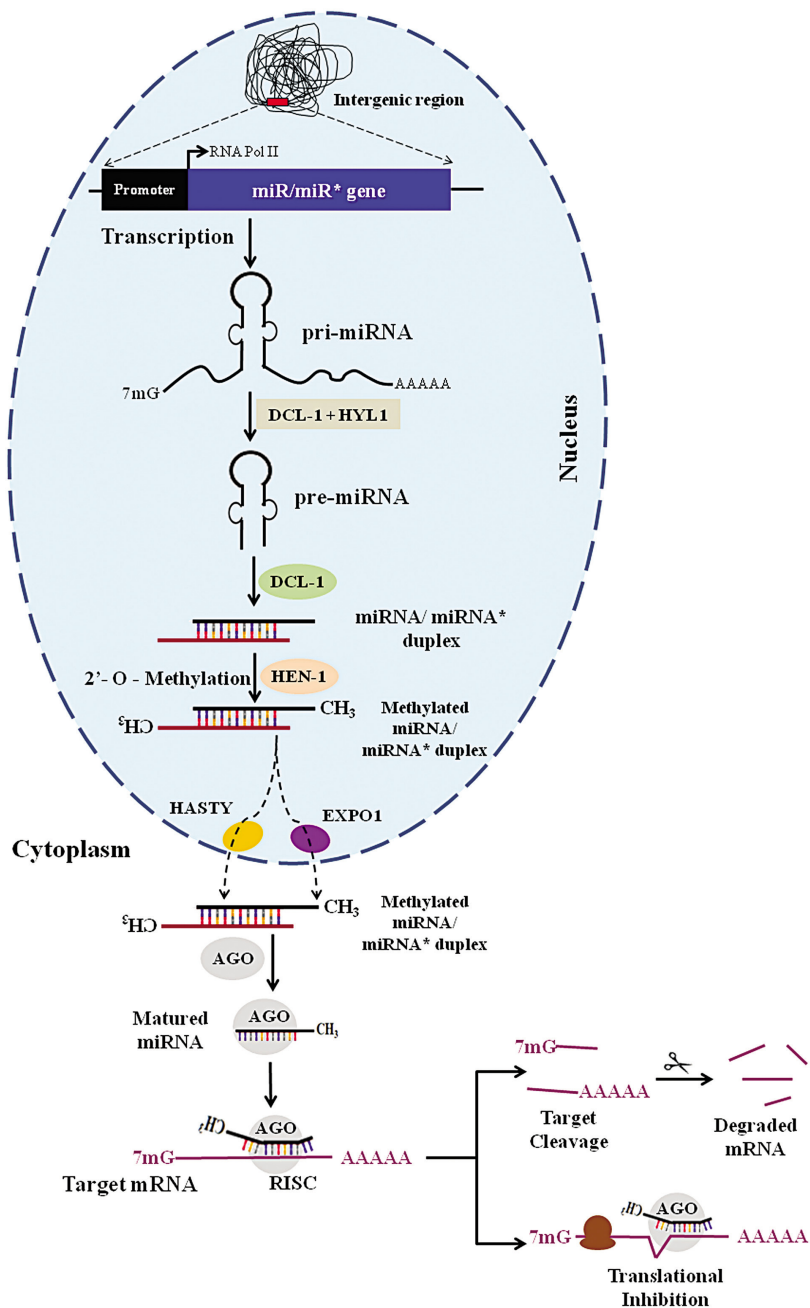
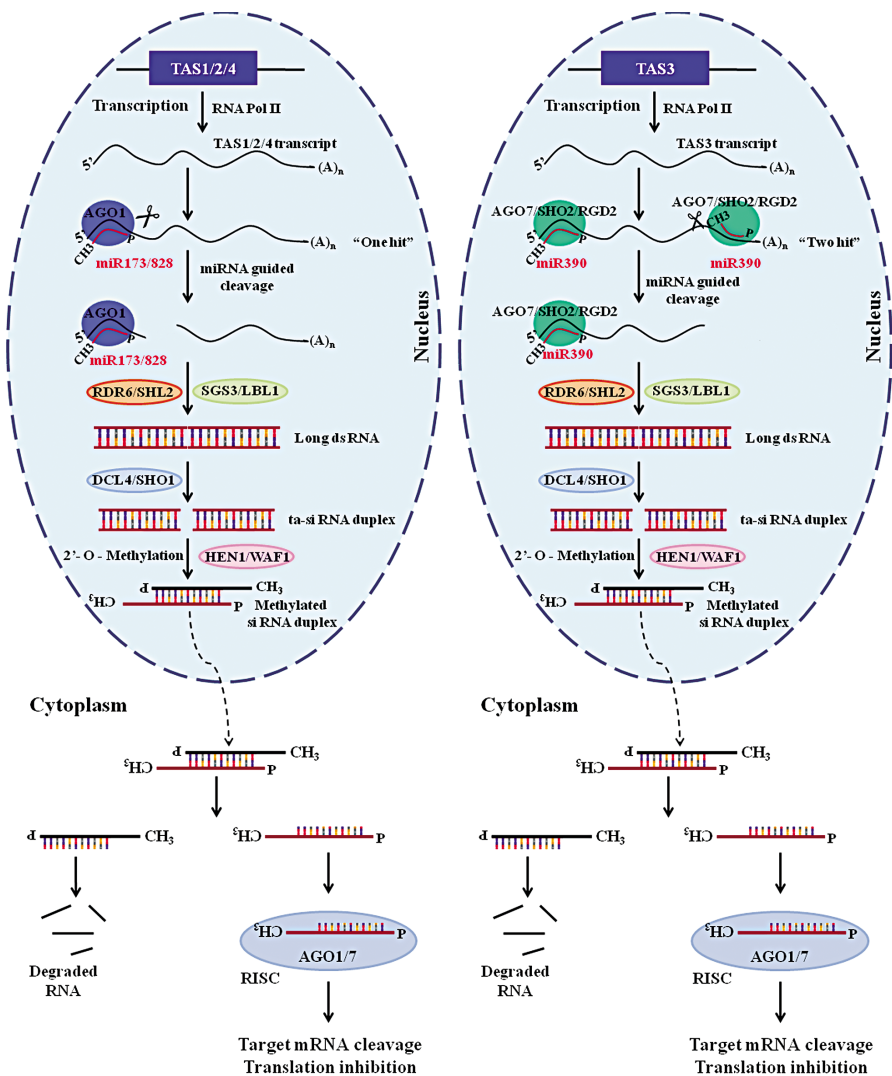


FIGURE 9.1 Biogenesis and mode of action of miRNA in plants.

siRNA, the most commonly documented two are retroelements and RNA virus replication (Khraiwesh et al., 2012). Though siRNAs are categorized into many subclasses all are produced by the action of a specific RDL-DCL protein complex (Kumar et al., 2018). In plants precursors of almost all siRNAs are long and double-stranded (Bartel & Bartel, 2003; Kim, 2005b) but the initiation of biogenesis is specific for each type of siRNA. To date, various researchers identified few types of siRNA precursors in plants. For example, ta-si RNAs are originated from non-coding regions, ra-siRNA originated from the heterochromatic locus. Guleria et al. (2011) found that single-stranded RNA (ssRNA) is utilized for particular siRNA via miRNA-mediated cleavage or RNA pol IV activity, and dsRNA is subsequently transcribed from single-stranded RNA precursors by RNA Dependent RNA polymerases (RDRs). The synthesized dsRNA is then split into two siRNAs by the action of the DCL protein, one of which is 21 nt long and the other is 24 nt long. The length of the synthesized siRNAs is determined by the specific enzymatic activity of the DCL proteins involved. A dsRNA molecule is often cleaved by several DCL proteins, that is why siRNAs of varying sizes are produced. After formation, siRNAs bind with AGO proteins to form RISCs, just like miRNAs, and similarly regulate target genes at the transcriptional and post-transcriptional levels via RNA-directed DNA methylation (RdDM). Several studies have proven the particular function of several DCL proteins in the production of siRNAs. In the case of ra-siRNAs dsRNAs are cleaved by DCL3 proteins whereas DCL 4 proteins catalyze the production of transacting siRNA. 21 nt long for ra-siRNA and 24 nt long siRNAs are produced by DCL3, whereas DCL4 helps in the biogenesis of 21 nt long ta-siRNA from the TAS RNA precursors (Figure 9.2), in association with the involvement of some other proteins namely RDR6, SGS3 and DRB 4 (ds RNA Binding Protein 4), etc. (Chowdhury, 2018). Biogenesis of siRNA and miRNA distinguished from each other in respect of dsRNA precursors and except this both the small RNA molecules are derived by the similar mechanisms, i.e., cleavage of dsRNA by the enzymatic action of Dicer-mediated cleavage (Khare et al., 2018).

### **9.5.1 MAJOR ROLES OF SMALL RNA AND SMALL RNA BASED TACTICS IN PLANTS**

Small RNAs are now widely known biomolecules that act as the riboregulator and control different gene regulation systems. In the context of plant science sRNAs are widely studied as they have a remarkable role in the generation



**FIGURE 9.2** Biogenesis and mode of action of siRNA in plants.

of stress responses. In addition to stress responses, some sRNAs are also identified to have significant roles in the regulation of normal biological function. Plants can alter their gene expression based on the situation of their habitat. Any stressful situation to plant, can dramatically alter the level of gene expression and also the pattern of gene regulation and such alteration



induces stress responses (Hirayama & Shinozaki, 2010; Muchate et al., 2016). To cope with the stressful situation, many plants have evolved some complicated morphological, molecular, and physiological regulatory systems. For a few decades, many researchers have utilized sRNA-based strategies to evaluate the stress resistance capacity of crop plants. Researchers have also screened and identified a large number of sRNAs encoded by plant genomes which impart significant contribution in protecting plants from various biotic and abiotic factors. To survive in adverse circumstances, plants employ diverse genetic, transcriptomic, and proteomic strategies. The majority of such studies have focused on *Arabidopsis thaliana*, *Glycine max*, *Gossypium raimondii*, *Hordeum vulgare*, *Medicago truncatula*, *Oryza sativa*, *Populus* sp., *Saccharum* sp., *Triticum turgidum*, *Vigna unguiculata*, and *Zea mays* (Noman & Aqeel, 2017). Though much exhaustive research has screened a huge number of sRNAs only a few of them have been recognized with detailed knowledge of their biological function:

1. In most eukaryotes, sRNAs are widely known for their gene regulatory action. By regulating gene expression, they can regulate many morphological and physiological events. Some of them regulate the gene expression downwardly and others are associated with upregulation of gene expression. Most of them execute such regulation by directing DNA to guide sequence elimination and remodeling of chromatin, whereas some of them act on RNA to guide cleavage and translational repression (Vaucheret, 2006).
2. Some of the sRNA molecules are also known for their capacity to silent a gene expression. Such short nucleotide sequences bind with specialized proteins to form Protein-RNA complexes which ultimately induce gene silencing. sRNA mediated gene silencing is executed through one of these three processes: (i) by decreasing the rate of transcription; (ii) by destabilization of primary transcript or active mRNA in the cell; or (iii) by decreasing the rate of translation (Mirlohi & He, 2016).
3. A large number of sRNAs have been identified in plants which play vital roles in the modulation of origin and growth of several vegetative and reproductive organs, including seeds (Singh et al., 2017).
4. Because of their mobility, these compounds are ideal riboregulators for non-cellular silencing. Non-cellular silencing can take place on a local or systemic level. Plants get benefit from these silencing strategies because these regulators can effectively reduce the harmful

effects of abiotic and biotic stressors and increase the beneficial effects of plant-microbe interactions.

5. In many studies sRNAs have been exhibited to regulate the level of secondary metabolites, proteins, and therapeutic substances in plants (de Fougerolles et al., 2007; Mello & Conte, 2004).

### 9.5.2 SMALL RNA-MEDIATED RNA SILENCING

The discovery of RNA silencing (RNAi) was a surprising and accidental observation. Jorgensen and his team in 1990 introduced an exogenous transgene in *Petunia* and attempted to increase the expression of a gene that codes for the enzyme Chalcone Synthase (catalyze the biogenesis of specific pigment compound) under the control of 35S promoter (Ali et al., 2010; Napoli et al., 1990). The experiment showed that the plants were unable to produce the usual deep purple flower and in spite of this often-produced variegated flowers with complete loss of color. Some workers considered it as co-suppression and explained that in *Petunia* both introduced transgene and added DNA interfere with the expression of endogenous loci (Pradhan et al., 2015). In the late 1990 Fire and colleagues conducted an experiment in *Caenorhabditis elegans* and showed that dsRNA can effectively execute silencing of endogenous genes through a sequence-specific mode of silencing (Fire et al., 1998). Later the process was coined as RNA interference or RNA silencing. Technically RNAi refers to the phenomenon that involves dsRNA mediated sequence-specific gene regulation and resulting in inhibition of either translation or transcription (Kamthan et al., 2015). The discovery of RNA interference has accelerated an enormous research effort in most of the fields of plant science and other relevant fields of science. In 1998, Andrew Fire and Craig Mello discovered that injecting dsRNA with both sense and antisense strands is more effective for silencing than injecting dsRNA with only one strand. Subsequent studies also confirmed the finding, which proved to be a defining moment in RNAi technology (Ali et al., 2010; Tabara et al., 1998). However, in the earlier mentioned discoveries are actually deals with the reporting of basic molecular mechanism related to gene silencing. In broad-spectrum RNA silencing is a non-cellular autonomous event as the silencing is initiated in one or few cells and progressively transmits RNA mediated sequence-specific suppression of homologous sequences in neighboring cell (Jorgensen et al., 1998; Voinnet & Baulcombe, 1997). Kalantidis et al. (2008) concluded that RNA silencing is transmitted from one plant cell to another via plasmodesmata, which is caused due to

cell-to-cell migration of a short-range silencing signal. According to several supporting investigations RNAi is also mechanistically related to various conserved RNA silencing pathways that are associated with cellular regulation of gene expression, protection of genome from mobile repetitive DNA sequences, retroelements, and transposons (Aravin et al., 2007; Baulcombe, 2002). The phenomenon of RNAi involves all types of non-coding sRNAs resulted from the cleavage product of dsRNA (Kamthan et al., 2015). In the case of local RNA silencing, the silencing signals can gradually spread from a cell to the group of its adjacent cells (Dunoyer et al., 2013; Marin-Gonzalez & Suarez-Lopez, 2012). On the contrary systematic silencing takes place over a certain distance and the silencing signal can spread from a cell to the distantly located cells. With the up-gradation of next-generation sequence technology a huge number of novel and conserved miRNAs and siRNAs have been identified in various plant species (Jover-Gil et al., 2004; Sun, 2012; Sunkar et al., 2012). Some of them were recognized through sequence analysis of constructed sRNA libraries, contrary others were predicted through *in silico* methods by utilizing recently accumulating sequence data. The gene silencing process needs some catalytic molecule that triggers the silencing of many homologous transcripts (Hinas et al., 2012; Hunter et al., 2006).

## **9.6 MODE OF ACTIONS OF SMALL RNAS IN PLANTS GROWN UNDER SALT STRESS CONDITION**

In most parts of the world, salinity appears to be a regular and recurrent crucial abiotic stress factor, which plays a vital role in restricting food production and is regarded as one of the major threats to biodiversity. Stress has simply referred to any kind of impairment in the growth and development of living organisms. Factors that generate stressful conditions are called stressors. In the context of plant science, the stressful situation causes delayed seed germination, insufficient growth, and decreased yield. Plant stress responses are fully reliant on accurate gene expression and correct gene regulation at several levels, including transcriptional, post-transcriptional, and post-translational (Mirlohi & He, 2016). Among the gene regulation, post-transcriptional regulation has been discovered to have a role in maintaining cellular homeostasis under stress conditions, as well as assisting cells in recovering from the detrimental effects of stress (Sunkar et al., 2012). Among the abiotic stresses, the most damaging one is salt-induced

stress. In a salt-sensitive plant slight increase of salt concentration from the critical value may lead to harmful effects on the structural integration and functioning of the plant. Disorganization of the cell membrane, formation of poisonous metabolites, development of reactive oxygen species, and suppression of photosynthetic processes are some of the most obvious and prevalent effects of salt on plant cells (Hasegawa et al., 2000). Alteration of cell membrane fluidity results in disruption of protein homeostasis, which in turn results in improper functioning of cellular processes in plants. Salinity can disturb the plant from seed germination to its maturation. In salt mediated stress conditions plants reprogramed their genetic organization at the transcription level in such a way that protective genes are up-regulated while the genes influence negatively are down-regulated. Modification of such a transcriptional system is one of the essential steps to trigger the adaptation processes under salinity (Gehan et al., 2015; Nakashima et al., 2014; Priest et al., 2014). The expression of numerous genes is modified in plants growing under salt-induced stressful conditions, and gene regulatory sRNAs should play a key role in the regulation of genes or gene-coded metabolites. Gradual research on sRNA mediated gene regulation has confirmed that under salinity the expressions of various miRNAs and siRNAs are altered while such modified sRNAs then alters the timing, location, and level of proteins expression of their target genes under salinity. The genetic model plant, i.e., *Arabidopsis thaliana* is readily amenable to molecular modification, thereby providing plant biology researchers with an excellent experimental system to validate the expression of abiotic stress tolerance phenotypes introduced through small RNA regulation and molecular modifications (Pegler et al., 2019). Many experimental evidence points to the fact that many miRNAs are closely linked to the salt response. According to the study of Liu et al. (2018), 12 miRNAs in *Arabidopsis*, miR156, miR158, miR159, miR165, miR167, miR168, miR169, miR171, miR319, miR393, miR394, and miR396 are elevated in reply to high salinity. In another plant *Populus euphratica* Olivier, 211 miRNAs have been identified and 162 of them were screened for salt response (Li et al., 2013). Different species of *Saccharum* also exhibited upregulation of different miRNAs (miR156, miR159, miR166, miR167, miR168, miR169, miR396, miR397, miR398, and miR528) under salinity (Bottino et al., 2013; Gentile et al., 2015; Kumar et al., 2018). In a study on *Vigna unguiculata*, 18 conserved miRNAs were reported from 16 miRNA families by using a comparative genomic approach, which is expressed in various ways under salinity (Paul et al., 2011). By analyzing sequencing data through various sophisticated bioinformatics tools, a huge number of

sRNAs of interest were identified and their targets were also identified. A correct prediction and effective validation of small RNAs target gene is highly beneficial to researchers in investigating how these sRNAs may be used to regulate various plant biological activities. Any sort of stress-sensitive genes, including salt-tolerance genes are intensively regulated by a complex system of transcription factors and sRNAs (Devi et al., 2013; Li et al., 2010, 2012). These miRNAs regulate several essential salt reactive genes and transcription factors (Table 9.1), and regulators were proven critical to plant salt tolerance (Fang et al., 2014; Mondal et al., 2018; Woodrow et al., 2012). Under any stressful condition all the developmental, metabolic, and adaptive processes in the plant, rely on the proper regulation of gene expression system, which may be achieved by either transcriptional regulation or post-transcriptional regulation.

### **9.6.1 TRANSCRIPTIONAL GENE SILENCING (TGS)**

The process of shutting down gene expression at the transcription level is referred to as transcriptional gene silencing (TGS). Usually, such transcriptional repressions are caused by chromatin modification or methylation of DNA. TGS in the plant is usually related to sRNA-directed DNA methylation (Sunkar & Zhu, 2004). Such silence needs the homology of sequences between the promoter and sRNA, which is thus also called homology-related gene silence (HDGS) (Kooter et al., 1999). To date, many miRNAs are reported to act on salt responsive genes and induce DNA methylation (Table 9.1). In some plants, many subsets of hc-siRNAs have been recognized that are intricately related to DNA methylation and in turn, considered to influence salt tolerance. In a study, it was found that the capability of salt tolerance in *Arabidopsis* was decreased when the DNA methylation at cytosine was blocked by the treatment of any agent such as 5-azacytidine (Boyko et al., 2010).

### **9.6.2 POST-TRANSCRIPTIONAL GENE SILENCING (PTGS)**

Post-transcriptional gene silencing (PTGS) is a process in which transcription remain unaffected, but the expression of the gene is lost as mRNA molecule become unstable. sRNAs generally function as sequence determinants for such gene silencing in plants. Distinct components have interacted during PTGS and components may differ for miRNA mediated PTGS and siRNA

**TABLE 9.1** List of mi-RNAs and Their Responses under Salt Stress

Plants	Small RNA	Status Under Salinity	Target Protein	References
<i>Agrostis stolonifera</i>	miR319	Overexpressed	TCP transcription factors; NAC	Zhou et al. (2013); Zhou & Luo (2014)
	miR396	Overexpressed	Growth-regulating factors (GRFs)	Gao et al. (2010); Mecchia et al. (2013)
	miR394	Overexpressed	F-box	Song et al. (2013)
<i>Arabidopsis thaliana</i>	miR156	Overexpressed	Squamosa promoter-binding protein-like 11	Liu et al. (2008)
	miR158	Overexpressed	Pentatricopeptide repeat-containing protein (PPR)	
	miR159	Overexpressed	MYB and TCP transcription factor	Chen et al. (2012); Liu et al. (2008)
	miR160	Overexpressed	Auxin response factor	Gutierrez et al. (2009); Sunkar et al. (2012)
	miR161	Overexpressed	ARGONAUTE1	Dolata et al. (2016)
	miR164	Overexpressed	NAC family gene	Amor et al. (2009)
	miR165	Overexpressed	Class III HD-ZIP transcription factor	Liu et al. (2008)
	miR166	Overexpressed	Squamosa promoter-binding protein-like 11	Amor et al. (2009)
	miR167	Overexpressed	Auxin response factor	Liu et al. (2008); Sunkar et al. (2012)
	miR168	Overexpressed	ARGONAUTE 1 (AGO1)	Liu et al. (2008)
	miR169	Overexpressed	CCAT-binding transcription factor (CBF-B/NF-YA) family protein	
	miR171	Overexpressed	Scarecrow-like transcription factor	
	miR173	Overexpressed	ARGONAUTE 1	Dolata et al. (2016)
	miR319	Overexpressed	TCP transcription factors	Liu et al. (2008)

**TABLE 9.1** (Continued)

Plants	Small RNA	Status Under Salinity	Target Protein	References
	miR393	Overexpressed	F-Box protein; basic helix-loop-helix (bHLH) transcription factor	Gao et al. (2011); Liu et al. (2008); Sunkar et al. (2012)
	miR394	Overexpressed	F-Box family protein	Liu et al. (2008)
	miR396	Overexpressed	Growth regulator factor 2; transcription factor; rhodanese like protein	
	miR397	Overexpressed	Laccase 2	
	miR398	Repressed	Inter Pro domain protein of unknown factor	
	miR408	Overexpressed	Laccase; plastocyanin	Liu et al. (2008); Ma et al. (2015); Sunkar & Zhu (2004)
<i>Cucumis sativus</i>	miR169	Overexpressed	CBF HAP2-like factor	Li et al. (2011)
	miR395	Overexpressed	ATP sulfurylase	Li et al. (2016)
	miR398	Repressed	Cu/Zn superoxide dismutase	
	miR399	Overexpressed	Protein PHR1-LIKE 1-like	
<i>Glycine max</i>	miR166	Overexpressed	SBP-like transcription factor	Li et al. (2011)
	miR169	Overexpressed	CBF HAP2-like factor	
	miR394	Repressed	F-box protein	
	miR1507	Overexpressed	NBS-LRR resistance protein	
<i>Gossypium hirsutum</i>	miRNVL5	Repressed	Zinc-finger domain-containing transcription factor	Gao et al. (2016)

**TABLE 9.1** (Continued)

Plants	Small RNA	Status Under Salinity	Target Protein	References
<i>Gossypium raimondii</i>	miR156	Overexpressed	Unindicated	Xie et al. (2015)
	miR157	Repressed	Unindicated	
	miR160	Repressed	Auxin response factor	
	miR166	Repressed	Unindicated	
	miR167	Repressed	Unindicated	
	miR172	Repressed	AP2-like ethylene-responsive transcription factor	
	miR394	Overexpressed	Unindicated	
	miR396	Repressed	Unindicated	
<i>Hordeum vulgare</i>	miR156	Overexpressed	Squamosa promoter-binding protein-like 11	Deng et al. (2015); Lv et al. (2012)
	miR168	Repressed	ARGONAUTE1 protein	
	miR171	Overexpressed	Protein FAN	Deng et al. (2015)
	miR444	Repressed	F-box family protein	Deng et al. (2015); Lv et al. (2012); Zeng et al. (2019)
	miR6213	Overexpressed	Unindicated	Deng et al. (2015); Lv et al. (2012)
	miR5048	Repressed	Unindicated	
<i>Lycopersicon esculentum</i>	miR169	Overexpressed	Nuclear factor Y subunit	Zhang et al. (2011)
<i>Medicago sativa</i>	miR166	Repressed	Class III homeodomain leucine zipper protein	Long et al. (2015)
	miR393	Repressed	F-box protein	
<i>Medicago truncatula</i>	miR390	Downregulated	Leucine-rich repeat receptor-like protein kinase	Lelandais-Brière et al. (2009)



**TABLE 9.1** (Continued)

Plants	Small RNA	Status Under Salinity	Target Protein	References
<i>Nicotiana tabbaccum</i>	miR159	Repressed	MYB transcription factor	Frazier et al. (2011)
	miR167	Downregulated	ARF transcription factor	
	miR396	Overexpressed	GRL transcription factors	
<i>Oryza sativa</i>	miR159	Repressed	MYB transcription factor	Barrera-Figueroa et al. (2012)
	miR160	Repressed	Auxin response factor (ARF)	
	miR164	Downregulated	Unindicated	Macovei & Tuteja (2012)
	miR169	Overexpressed	Nuclear transcription factor Y subunit CCAAT-binding transcription factor	Archak & Nagaraju (2007); Li et al. (2010); Zhao et al. (2009)
	miR393	Overexpressed	TIR1, AFB2, AFB3, F-box domain, MYB family TF	Liu et al. (2008); Xia et al. (2012)
	miR394	Down-regulated	F-box protein	Macovei & Tuteja (2012)
	miR396	Repressed	Growth regulating factor	Gao et al. (2011); Yuan et al. (2019)
	miR398	Overexpressed	Copper/zinc superoxide dismutase 1, copper/zinc superoxide dismutase 2, and copper chaperone for SOD	Li et al. (2010); Lu et al. (2011)
	miR408	Repressed	Blue copper protein precursor; peptide chain release factor	Li et al. (2010); Macovei & Tuteja (2012); Mutum et al. (2013)
	miR528	Repressed	L-ascorbate oxidase	Yuan et al. (2015)
	miR809	Repressed	Glutaredoxin 2, putative; Glutaredoxin subgroup II; PPR repeat-containing protein	Barrera-Figueroa et al. (2012); Li et al. (2010)
	miR820	Repressed	NAC domain-containing protein; domains rearranged methyltransferase 2; Plasma membrane intrinsic protein 1–5	Nosaka et al. (2012); Sahu et al. (2013); Sharma et al. (2015)
	miR2871	Overexpressed	Glycosyltransferase family protein	Barrera-Figueroa et al. (2012)

**TABLE 9.1** (Continued)

Plants	Small RNA	Status Under Salinity	Target Protein	References
<i>Panicum virgatum</i>	miR156	Upregulated	Squamosa promoter-binding protein-like	Sun et al. (2012)
	miR157	Upregulated	Squamosa promoter-binding protein-like	
	miR159	Upregulated	MYB transcription factor	
	miR162	Upregulated	DICER-LIKE 1 (DCL1)	
	miR395	Repressed	ATP sulfurylase	
	miR396	Overexpressed	GRL transcription factors	
	miR397	Repressed	cDNA l-ascorbate oxidase precursor	
	miR399	Repressed	ATP-dependent RNA helicase	
<i>Phaseolus vulgaris</i>	miR2118	Overexpressed	APS-reductase	Arenas-Huerta et al. (2009)
<i>Populus euphratica</i>	miR1446	Repressed	Gibberellin response modulator-like protein	Qiu et al. (2011)
	miR1447	Overexpressed	ATP-binding cassette transport protein	
	miR1448	Repressed	Unindicated	
<i>Populus trichocarpa</i>	miR156	Repressed	POPTR_0007s01030	Li et al. (2013)
	miR164	Overexpressed	POPTR_0007s08420	
	miR172	Overexpressed	POPTR_0008s04490	
	miR474	Repressed	PPR; protein kinase; kinesin; leucine-rich repeat	Zhou et al. (2012)
	miR482	Overexpressed	TIR-NBS-LRR resistance protein	Lu et al. (2008)
	miR530	Repressed	F-box domain-containing protein	
	miR1444	Repressed	POPTR_0001s39950	
	miR1445	Repressed	Unindicated	
	miR1711	Repressed	Unindicated	

**TABLE 9.1** (Continued)

Plants	Small RNA	Status Under Salinity	Target Protein	References
<i>Raphanus sativus</i>	miR156	Overexpressed	Squamosa promoter-binding protein like	Sun et al. (2015)
	miR160	Repressed	Auxin response factor	
	miR164	Overexpressed	NAC domain-containing protein	
	miR169	Overexpressed	Nuclear transcription factor Y	
	miR172	Overexpressed	AP2-like ethylene-responsive transcription factor	
	miR396	Overexpressed	Transcription factor bHLH74	
<i>Saccharum sp.</i>	miR156	Overexpressed	Squamosa binding protein-domain protein	Bottino et al. (2013)
	miR159	Overexpressed	GAMyb protein	
	miR166	Overexpressed	Class III HD-zip protein 4	
	miR167	Overexpressed	Auxin response factor	
	miR168	Overexpressed	Argonaute 1	
	miR169	Overexpressed	HAP12-CCAAT-box transcription factor complex	
	miR396	Overexpressed	Growth-regulating factor 1	
	miR397	Overexpressed	Laccase-10 precursor	
	miR398	Overexpressed	Selenium binding protein	
	miR528	Overexpressed	Putative laccase	
	miR160	Repressed	Auxin response factor	Khan et al. (2014)
	miR171	Downregulated	Gibberellic acid insensitive (GAI)	
<i>Setaria italica</i>	miR162	Overexpressed	Dicer-like 1 (DCL1)	Sun et al. (2012)

**TABLE 9.1** (Continued)

Plants	Small RNA	Status Under Salinity	Target Protein	References
<i>Solanum linnaeanum</i>	miR156	Repressed	Squamosa promoter-binding protein	Zhuang et al. (2014)
	miR162	Repressed	Ribonuclease 3-like protein 3	
	miR164	Overexpressed	Lipase-related	
	miR167	Repressed	Annexin 1	
	miR319	Repressed	TCP transcription factor	
	miR397	Overexpressed	Laccase	
	miR399	Repressed	Unindicated	
	miR5300	Repressed	Nucleotide-binding site-leucine-rich repeat	
<i>Triticum aestivum</i>	miR156	Repressed	Squamosa promoter-binding protein	Akdogan et al. (2016); Pandey et al. (2014); Xin et al. (2010)
	miR164	Repressed	NAC transcription factor	
	miR167	Repressed	Putative auxin response transcription factor (ARF)	
	miR171	Repressed	Scarecrow-like transcription factor	
	miR396	Repressed	Growth-regulating factor	
	miR160	Overexpressed	Auxin response factor	
	miR165	Repressed	Unindicated	
	miR399	Overexpressed	ATP-dependent RNA helicase	
	miR408	Overexpressed	Peptide chain release factor; plantacyanin	
	miR1029	Repressed	Unindicated	
<i>Vigna unguiculata</i>	miR156	Overexpressed	Squamosa promoter-binding protein	Gupta et al. (2014) Lu & Yang (2010); Paul et al. (2011)
			Rho-related protein racG	
	miR157	Overexpressed	Squamosa promoter-binding protein	
	miR160	Overexpressed	Auxin response factor (ARF)	

**TABLE 9.1** (Continued)

Plants	Small RNA	Status Under Salinity	Target Protein	References
<i>Zea mays</i>	miR162	Overexpressed	RNAseIII CAF protein	Paul et al. (2011)
	miR169	Overexpressed	CCAAT-binding transcription factor (CBF)	
	miR408	Overexpressed	Basic blue copper protein/Plantacyanin	
	miR156	Repressed	SBP-domain protein 6	Ding et al. (2009); Sun et al. (2015)
	miR395	Overexpressed	ATP sulfurylase	
	miR396	Repressed	Cytochrome oxidase subunit-I	
	miR162	Overexpressed	Endoribonuclease dicer	
	miR159	Overexpressed	MADS-box interactor	Ding et al. (2009)
	miR160	Overexpressed	Two-component response regulator APR11	
	miR164	Repressed	NAC domain protein NAC I	Li et al. (2012); Sun et al. (2015)
	miR166	Overexpressed	Homeodomain leucine zipper protein (HD-ZIP)	Sun et al. (2015)
	miR167	Repressed	Auxin Response Factor	
	miR168	Co-regulated like a feedback loop	ARGONAUTE 1 (AGO1)	Kong et al. (2010); Sun et al. (2015); Vaucheret (2008)
	miR169	Repressed	Nuclear transcription factor Y sub-unit	Luan et al. (2014)
	miR171	Overexpressed or repressed	Spry-2	Ding et al. (2009); Kong et al. (2010)

mediated PTGS. miRNA precursors are processed by dicer like 1 (DCL1) into mature miRNAs, whereas siRNAs from transgenes, viruses, and endogenous transcripts are generated by other DCLs (Fei et al., 2013; Fukudome et al., 2011; Kurihara & Watanabe, 2004). Several investigations concluded that target genes of miRNAs in plants are linked with many developmental processes, as a result, any change in miRNAs might cause anomalies in plant growth and development. In *Arabidopsis*, it was demonstrated that the splitting of miRNAs from dsRNA is directed by some other stress-responsive miRNAs and further studies with high-throughput sequencing has screened such stress-responsive miRNAs as well as the cleavage sites of these miRNAs (Jagadeeswaran et al., 2009; Llave et al., 2002). For example, it was reported that the expression level of miR398 was decreased under salt-induced stress (Bouche, 2010). It has been concluded that PTGS is quite essential to check the total “transcript dynamics” under stress conditions (Joshi et al., 2017). In general salt resistance in plants is achieved by multiple genes, and the genes are also regulated at multiple levels due to salinity. Under salinity, plants activate all the stress-responsive genes as well as stress-responsive sRNAs to combat stressful situations and try to maintain structural, biochemical, and physiological activities properly (Covarrubias & Reyes, 2010). Several salt stress-responsive novels and identified miRNAs have been identified from many plants. In most situations, miRNAs suppress the expression of endogenous genes by directing the cleavage of their target mRNAs (Mallory & Vaucheret, 2006). Expression of miRNAs has been found to alter in a dose-dependent manner by salinity. Frazier et al. (2011) showed that salinity stress can change the level of expression of miRNA from 6.86-fold down-regulation to 616.57-fold upregulation in *Nicotiana*. Extensive investigations on miRNA and post-transcriptional gene regulation in plants have explored the fact that none of the target genes/proteins of conserved miRNAs is directly associated with salt tolerance; however, they have control over the expression of genes that are associated with salt tolerance (Sunkar et al., 2012). The impact of miRNAs on the salt responsive gene depends on their affinity, location, and abundance. Manavella et al. (2019) opined that whole machinery associated with the biogenesis of miRNA can be turned down through the feedback regulatory mechanism. Many siRNAs have recently come into the limelight as important players in plant stress responses; these are more diverse than miRNA. Among the identified siRNAs most widely studied subsets are ta-siRNAs, nat-siRNAs, and hc-siRNAs (Ku et al., 2015). The mode of actions of different siRNA is quite different. Plants have two major endogenous siRNA-mediated regulations, hc-siRNAs mediated

regulation and ta-siRNAs mediated regulation. These can be introduced exogenously into the plants to regulate stress responses or sustain growth and development under stressful situations (Guleria et al., 2011). The ta-siRNAs bound to the AGO1 complex joins to specific sites in the target mRNA and executes gene silencing either through mRNA degradation or DNA methylation, or inhibition of synthesis of specific functional proteins at the post-transcriptional levels (Dutta et al., 2020). To date almost 80% of identified siRNAs are hc-siRNAs and more than thousands of them were reported to present in *Arabidopsis* (Sanan-Mishra et al., 2021). Such siRNAs are reported to participate both in the modification of histone proteins and suppression of gene expression at the transcription level (Dutta et al., 2020). Besides this, some siRNAs have also been found in several plants and are effective in stress resistance. In spite of the recognition of the multiple importance of siRNAs in stress response in plants as well as in growth and development, a lot of things still remain undiscovered and unraveled, especially the information regarding their biogenesis, functions, and mode of action. Under stressful conditions, nat-siRNAs in the majority of plants have been reported to generate nat-siRNA for the regulation of stress-responsive genes. By conducting gradual experiments on *Arabidopsis* growing under salinity, researchers found that nat-siRNAs, are generated from a specialized cis-NAT gene duo, made up of SRO5 and P5CDH genes and further research has shown that nat-siRNAs are important in protecting plants from osmotic and oxidative stress (Borsani et al., 2005). Further findings concluded that the pyrroline-5-carboxylate dehydrogenase (P5CDH) gene encodes an enzyme that catalyzes proline degradation, while the salt stress-inducible gene (SRO5) is involved in managing ROS production. Introduction of the SRO5 gene generates dsRNA sequences in SRO5-P5CDH overlapping juncture (Deuschle et al., 2004; Dutta et al., 2020). The hc-siRNAs also play a key role in gene regulation when these are methylated by HEN1 and in all cases, they act as a component of a complex structure similar to RISC, named as RNA-induced transcriptional silencing complex (RITS) (Sanan-Mishra et al., 2021). In *Triticum*, the pool of stress-related siRNAs was reported; among them siRNA002061\_0636\_3054.1, siRNA005047\_0654\_1904.1 and siRNA007927\_0100\_2975.1 are regulated in a descending manner by salt-induced stress (Yao et al., 2010). Another endogenous siRNA has been reported in *Craterostigma plantagineum* which is induced during dehydration and similar stress conditions and can regulate desiccation tolerance (Furini et al., 1997). The discovery of siRNAs and RNAi has opened a new era that might introduce a novel tool for crop improvement, especially in the

development of stress-resistant crops. The design and engineering of siRNA carriers in plants have gained significant momentum in the recent era, as a result of the accumulation of predictable and promising molecular targets. Due to the continuous efforts of researchers, a large number of siRNAs have been discovered and many more will be identified in the near future by using stress treated plant samples. *Arabidopsis thaliana* the genetic model plant is readily responsive to molecular modification, thereby provide plant biology researchers with an outstanding experimental system to corroborate which introduced molecular modifications mediate the expression of abiotic stress tolerance phenotypes (Pegler et al., 2019). Intracellular RNA silencing technique also provide some evidence of sRNA mediated transfer of silencing state between plant cells (Voinnet & Baulcombe, 1997; Voinnet et al., 1998). The signals of gene silencing in plants can move from one cell to the adjacent cells through plasmodesmata whereas the signal moves through the vascular system for a greater distance (Hamilton et al., 2002).

## **9.7 COMPUTATIONAL TOOLS, TECHNIQUES, AND DATABASE AVAILABLE FOR SRNA AND ITS TARGET PREDICTION**

In this age, computational techniques have integrated with all fields of biological sciences and are commonly known as Computational Biology. By using DNAs, RNAs, and Proteins, many cellular, biochemical, and physiological phenomenon is clearly explained. The research field of Molecular Biology has experienced a noticeable shift in its philosophy in recent times mostly because of the growing evidence of gene regulation by sRNA molecules in both plants and animals. Researchers of different corners of the earth working on plants and animals have greatly benefitted from the strategy of high-throughput sequencing technology and the progressive advancement of this strategy provide an unprecedented level of sRNA profiling which seems to have arrived at a plateau from which researchers are capable to characterize these molecules mechanistically and/or functionally (Park & Shin, 2014). Due to such an advantage, a huge number of sRNAs, especially miRNAs have been identified in many plants and many of them are unique and non-conserved (Cuperus et al., 2011; Fahlgren et al., 2007). With this study, some of the researchers have set their attention to highly conserved miRNAs, which are associated with fundamental cellular events and plant evolution (Willmann & Poethig, 2007). Extensive research by using this technology researchers have uncovered the fact that conserved miRNAs



are involved in the regulation of targets of those miRNAs which are not widely conserved (Debernardi et al., 2012; Zhao et al., 2016). Accelerated bioinformatics and computational biology enable the opportunity to study molecular mechanisms related to various stress responses in plants. For a proper understanding of the molecular mechanism of plant stress responses under salinity, miRNA-based, and si-RNA based computational strategies are widely used. Direct cloning, forward genetics, and bioinformatics are the most common methods for identifying and screening miRNAs, and these methods have resulted in the discovery of a vast number of miRNAs. There are several tools and techniques that are available to analyze other kinds of sRNAs and also to study the gene regulations control by them. However, still, the identification of sRNA molecules remains quite a difficult task. The first step for identifying small RNAs is to obtain a collection of sequences that might contain small RNA transcripts and the genome sequences (if available), transcriptome data, and expressed sequence tags (ESTs) can all be used for discovering small RNA genes (Li et al., 2012). To date, in terms of reliability and sensitivity, the best choice for obtaining a sequence of small RNAs is the sRNA library. By comparative analysis of newly found sequences to those in databases and detecting overlap in genomic location between the new data and databases, miRNAs can be classified into several categories. The unannotated sequences are utilized by the self-developed program Mireap to predict new miRNAs (Fu et al., 2017). An in-depth study of the miRNA-mRNA regulatory network provides knowledge about post-transcriptional fine adjustment of gene expression. However, *in silico* predictions about the interaction between miRNA and mRNA do not address the specific transcriptomic situation of a particular biological system and are often influenced by false positive (Meyer et al., 2014). Genome sequences can also be exploited to find small RNA genes, and they offer the possibility of finding all small RNA genes, but a high rate of false positives needs to be considered (Li et al., 2012). In addition to the NCBI genome database, a few data centers are also helpful to find small RNA genes or small RNA sequences. The plant microRNA database (PMRD, <http://bioinformatics.cau.edu.cn/PMRD>) now contains over 10,000 miRNAs from 121 plant species (Zhang et al., 2010). Bioinformatics techniques like EST, GSS (genome survey sequences), and many others are used to discover miRNAs, including probable miRNA precursors capable of forming a hairpin-like secondary structure (Sunkar & Jagadeeswaran, 2008; Zhang et al., 2005) (Tables 9.2–9.4).

**TABLE 9.2** Useful Tools for microRNA Prediction in Plants

Program	Website	References
miRAlign	<a href="http://bioinfo.au.tsinghua.edu.cn/miralign/">http://bioinfo.au.tsinghua.edu.cn/miralign/</a>	Wang et al. (2005)
miRFinder	<a href="http://sundarlab.ucdavis.edu/mirna/">http://sundarlab.ucdavis.edu/mirna/</a>	Huang et al. (2007)
Mireval	<a href="http://tagc.univ-mrs.fr/mireval/">http://tagc.univ-mrs.fr/mireval/</a>	Ritchie et al. (2008)
miRNEST	<a href="http://lemur.amu.edu.pl/share/php/mirnest/home.php">http://lemur.amu.edu.pl/share/php/mirnest/home.php</a>	Szczęśniak et al. (2012)
mirExplorer	<a href="http://biocenter.sysu.edu.cn/mir/">http://biocenter.sysu.edu.cn/mir/</a>	Guan et al. (2011)
MiRPara	<a href="http://www.whiov.ac.cn/bioinformatics/mirpara">http://www.whiov.ac.cn/bioinformatics/mirpara</a>	Wu et al. (2011)
findMiRNA	<a href="http://www.bioinformatics.org/mirfinder/">http://www.bioinformatics.org/mirfinder/</a>	Adai et al. (2005)
Micro-harvester	<a href="http://ab.inf.uni-tuebingen.de/brisbane/tb/index.php">http://ab.inf.uni-tuebingen.de/brisbane/tb/index.php</a>	Dezulian et al. (2006)
MicroPC	<a href="http://www.biotech.or.th/isl/micropc">http://www.biotech.or.th/isl/micropc</a>	Mhuantong & Wichadakul (2009)
SplamiR	<a href="http://www.uni-jena.de/SplamiR.html">http://www.uni-jena.de/SplamiR.html</a>	Thieme et al. (2011)
miRTour	<a href="http://bio2server.bioinfo.uni-plovdiv.bg/miRTour/">http://bio2server.bioinfo.uni-plovdiv.bg/miRTour/</a>	Milev et al. (2011)

**TABLE 9.3** Useful Tools for microRNA Target Prediction in Plants

Program	Website	References
MicroInspector	<a href="http://mirna.imbb.forth.gr/microinspector/">http://mirna.imbb.forth.gr/microinspector/</a>	Rusinov et al. (2005)
miRBase	<a href="http://www.mirbase.org/">http://www.mirbase.org/</a>	Griffiths-Jones et al. (2006)
miTarget	<a href="http://cbit.snu.ac.kr/~miTarget/">http://cbit.snu.ac.kr/~miTarget/</a>	Kim et al. (2006)
TarBase	<a href="http://www.diana.pcbi.upenn.edu/tarbase">http://www.diana.pcbi.upenn.edu/tarbase</a>	Sethupathy et al. (2006)
miRU	<a href="http://bioinfo3.noble.org/miRU.htm">http://bioinfo3.noble.org/miRU.htm</a>	Zhang (2005)
findmiRNA	<a href="http://sundarlab.ucdavis.edu/mirna/">http://sundarlab.ucdavis.edu/mirna/</a>	Adai et al. (2005)
MicrPC	<a href="http://www.biotech.or.th/isl/micropc">http://www.biotech.or.th/isl/micropc</a>	Mhuantong & Wichadakul (2009)
TAPIR	<a href="http://bioinformatics.psb.ugent.be/webtools/tapir/">http://bioinformatics.psb.ugent.be/webtools/tapir/</a>	Bonnet et al. (2010)
Target-align	<a href="http://www.leonxie.com/targetAlign.php">http://www.leonxie.com/targetAlign.php</a>	Xie & Zhang (2010)
miRTour	<a href="http://bio2server.bioinfo.uni-plovdiv.bg/miRTour/">http://bio2server.bioinfo.uni-plovdiv.bg/miRTour/</a>	Milev et al. (2011)

**TABLE 9.4** Useful Tools for siRNA in Plants

Programs	Links	References
ASRP	<a href="http://asrp.cgrb.oregonstate.edu/">http://asrp.cgrb.oregonstate.edu/</a>	Gustason et al. (2005)
sIR	<a href="http://biotools.swmed.edu/siRNA">http://biotools.swmed.edu/siRNA</a>	Shah et al. (2007)
Specificity-Server	<a href="http://informatics-eskitis.griffith.edu.au/SpecificityServer">http://informatics-eskitis.griffith.edu.au/SpecificityServer</a>	Chalk & Sonnhammer (2008)
AsiDesigner	<a href="http://sysbio.kribb.re.kr/AsiDesigner/">http://sysbio.kribb.re.kr/AsiDesigner/</a>	Park et al. (2008)
siDirect 2.0	<a href="http://siDirect2.RNAi.jp">http://siDirect2.RNAi.jp</a>	Naito et al. (2009)
DEQOR	<a href="http://bioinformatics.age.mpg.de/bioinformatics/DEQOR.html">http://bioinformatics.age.mpg.de/bioinformatics/DEQOR.html</a>	Henschel et al. (2004)
DSIR	<a href="http://biodev.cea.fr/DSIR/DSIR.html">http://biodev.cea.fr/DSIR/DSIR.html</a>	Vert et al. (2006)
NEXT-RNAi	<a href="http://b110-wiki.dkfz.de/signaling/wiki/display/nextrnai/NEXT-RNAi">http://b110-wiki.dkfz.de/signaling/wiki/display/nextrnai/NEXT-RNAi</a>	Horn et al. (2010)
Oligo Walk	<a href="http://rna.urmc.rochester.edu/cgi-bin/serverexe/oligowalk/oligowalk_form.cgi">http://rna.urmc.rochester.edu/cgi-bin/serverexe/oligowalk/oligowalk_form.cgi</a>	Lu & Mathews (2008)
OptiRNA	<a href="http://optirna.unl.edu/">http://optirna.unl.edu/</a>	Ladunga (2007)
OptiRNAi 2.0	<a href="http://rnaï.nci.nih.gov/">http://rnaï.nci.nih.gov/</a>	Cui et al. (2004)
MPSS	<a href="http://mpss.udel.edu">http://mpss.udel.edu</a>	Nakano et al. (2006)
Sfold2.2(Srna)	<a href="http://sfold.wadsworth.org/cgi-bin/sirna.pl">http://sfold.wadsworth.org/cgi-bin/sirna.pl</a>	Ding et al. (2004)
siDRM	<a href="http://siRecords.umn.edu/siDRM/">http://siRecords.umn.edu/siDRM/</a>	Gong et al. (2008)
siRNA selector	<a href="http://sirna.wi.mit.edu/">http://sirna.wi.mit.edu/</a>	Yuan et al. (2004)

The profile of DNA methylation and small RNA profiling will reveal genes or regions that are influenced by miRNA/siRNA-mediated DNA methylation and such profiling could help to explain how stress effects are epigenetically passed down through the generations (Khraiwesh et al., 2012).

## 9.8 APPLICATION AND ACHIEVEMENTS OF SMALL RNA-BASED STRATEGIES FOR THE BETTERMENT OF AGRICULTURE AND PLANT SCIENCE

Manipulation of small RNA-guided gene regulation in stress-sensitive plants to increase stress tolerance is a unique and viable approach. Improving salt tolerance in plants necessitates a thorough knowledge of gene transcriptional and post-transcriptional regulation by sRNAs under salt-induced stress conditions. Small RNA based gene silencing or RNAi technology has effectively been employed in the identification and functional assessment of several genes within a genome (Younis et al., 2014). RNAi is used by several groups of plants, including crop plants to reduce the expression of exogenous or endogenous mRNA by RISCs (Fire et al., 1998). RISCs regulate

gene expression in the cytoplasm by either degrading mRNAs or inhibiting their translation (Huntzinger & Izaurralde, 2011; Zhang et al., 2019). Now a days small RNA molecules, more specifically miRNAs are used as weapons of superpower to combat abiotic stresses. Plant breeders and plant genetic engineers develop a huge number of stress-resistant cultivars by using the concept of RNAi technology. The yield of any plants growing under stress conditions such as salinity conjointly depends on the survival of the plant as well as the phenological and developmental plasticity of the plant. Such miRNAs do not directly control the growth and development of plant, but by influencing through a miRNA-target gene network, they indirectly regulate the same. Emerging evidence also confirms the impact of miRNAs on the cellular and physiological events of many plants, and as a result, it is considered one of the valuable entities for crop improvement by plant genetic engineers. Appropriate modulation of target genes/proteins can help overcome PTGS, resulting in increased expression in treated plants.

Proper characterization of the functional feature of sRNAs together with a whole-genome sequence of plant species of agriculture relevance is also beneficial for transgenesis practice (Kumar et al., 2018). In addition, such studies on sRNA mediated regulatory networks will also provide a genetic basis for future research, including the research related to salt stress responses (Xu et al., 2019). Since few sRNA-based strategies are widely used in the study of seed germination in a normal and stressed conditions. By applying the RNAi approach seed germination process has improved in many plants through the down-regulation of lysine catabolism specifically when an elevated level of lysine negatively influences the events of germination (Zhu & Galili, 2004). In addition to gathering knowledge regulation of genes related to the development of organ and cellular metabolism sRNA-based strategy has also been implicated to enrich the understanding of constitutive silencing of gene-poor, repetitive sections of eukaryotic genomes, which are typically clustered at centromeres and telomeres (Johnson & Straight, 2017). In plants and some animals, miRNAs and many types of siRNAs execute post-transcriptional RNA interference (RNAi) as an adaptive response (Xie et al., 2004). RNAi technology has now become a powerful tool for crop improvement as it: (i) provides high throughput genotype-phenotype mapping in the plant; (ii) can also be implemented for rapid identification of biochemical pathways related to biogenesis phytoconstituents; and (iii) can also enable as an eco-friendly green alternative to conventional chemical pesticides by imparting resistance against bacteria, fungi, insects, and diverse plant viruses (Zhang et al., 2020).

## 9.9 FUTURE PERSPECTIVE

The wide acceptance of sRNA mediated strategy and progressive development of allied technology enable researchers to explore many underlying mechanisms of gene expression. The construction of different kinds of sRNAs and the up gradation of bioinformatics has allowed a new perspective of biological science. Such research enables us to develop advanced cultivars of crops and other beneficial plants (Kamthan et al., 2015). Recent findings showed that plant miRNA and phased siRNA are expressed spatially and temporally and thus manipulation of these sRNA molecules in a specific tissue and at a particular stage of development, can precisely target the trait(s) for improvement (Zhang et al., 2019). Another study has confirmed that sRNA mediated gene silencing efficiently initiates and drive the repression of both class 1 transposable elements (RNA) and class 2 transposable elements (RNA) (Wang et al., 2020). Application of CRISP/Cas 9 technology-based approach for cloning of miRNA genes and use of artificial miRNA in the study of genomics provide a promising advantage in the field of stress biology (Budak & Zhang, 2017; Zhou & Luo, 2013).

However, some drawbacks and negative feedback have emerged out due to various apparent discrepancies where target mRNAs were abundantly remained unaffected by miRNA overexpression, but target proteins were remarkably down-regulated (Aukerman & Sakai, 2003; Bari et al., 2006; Gandikota et al., 2007). Another vital issue is that sRNA-based technology still has not achieved the goal because of lack of high-performance testing and also due to certain features of such sRNAs like their tiny size, presence of sRNAs within genomic samples just like background noise or junk DNA (Budak & Zhang, 2017; Grobhans & Filipowicz, 2008; Kawaji & Hayashizaki, 2008). In addition, cell-specific and Spatio-temporal regulation of sRNA is also an obstacle for proper understanding of small RNA biology (Park & Shin, 2014). Progressive studies of the drawbacks and enhanced knowledge of sRNA functioning will be helpful to improve various structural and agronomic traits along with resistance against salinity and similar stressors.

## 9.10 CONCLUSION

Plants are frequently exposed to many environmental cues like biotic and abiotic stressors. To alleviate the negative influence of such stressors a strict transcriptomic reprogramming is very necessary for the survival of

stress-sensitive plants. It is well accepted among interdisciplinary researchers that there is an urgent need to address the negative impacts of unfavorable environmental constraints. Unfavorable environmental factors like high salinity are extremely decisive to the ecosystem and its components as these factors have a multitudinous negative impact on plants. For a few decades, scientists have discovered many strategies and tactics to protect the plants from salt-induced stress. Among the strategies to protect plants from salt stress, Small RNA-based technologies are much appreciated by many plant scientists. Since their initial identification in *Arabidopsis*, sRNAs have been, repeatedly demonstrated to be a key regulator of gene expression across all phases of plant development in mediating a defense response against several environmental stresses including soil salinity (Liu et al., 2008; Sunkar & Zhu, 2004). Plant miRNAs and siRNA generally execute their biological function via negative regulation of specific targets, and there is no space for doubt that they are the vital player in the regulation of stress tolerance and to achieve stress-resistant cultivars they are the striker. The fact is further supported by numerous findings related to plant responses under salinity and involvement of sRNA to influence stress response, which was published since the invention of RNA interference in the late 1990s. However, the field of sRNA biology is a big ocean of knowledge, and only a few drops have been achieved by the advanced technology of the present era and the complete dedication of researchers. Betterment of technology more involvement of researchers will surely uncover many underlying phenomena of plant metabolism and stress response in the near future.

## KEYWORDS

- **glycophyte**
- **halophyte**
- **microRNAs**
- **RNA interference**
- **salinity**
- **salt resistance**
- **small RNA**
- **trans-acting siRNA**

## REFERENCES

- Achkar, N. P., Cambiagno, D. A., & Manavella, P. A., (2016). miRNA biogenesis: A dynamic pathway. *Trends in Plant Science*, 21(12), 1034–1044. <https://doi.org/10.1038/nrm2632>.
- Adai, A., Johnson, C., Mlotshwa, S., Archer-Evans, S., Manocha, V., Vance, V., & Sundaresan, V., (2005). Computational prediction of miRNAs in *Arabidopsis thaliana*. *Genome Research*, 15(1), 78–91. <http://dx.doi.org/10.1101/gr.2908205>.
- Agarwal, P. K., Shukla, P. S., Gupta, K., & Jha, B., (2013). Bioengineering for salinity tolerance in plants: State of the art. *Molecular Biotechnology*, 54(1), 102–123. <https://doi.org/10.1007/s12033-012-9538-3>.
- Agrawal, N., Dasaradhi, P., Mohammed, A., Malhotra, P., Bhatnagar, R. K., & Mukherjee, S. K., (2003). RNA interference: Biology, mechanism, and applications. *Microbiology and Molecular Biology Reviews*, 67(4), 657–685. <https://doi.org/10.1128/MMBR.67.4.657-685.2003>.
- Akdogan, G., Tufekci, E. D., Uranbey, S., & Unver, T., (2016). miRNA-based drought regulation in wheat. *Functional & Integrative Genomics*, 16(3), 221–233. <https://doi.org/10.1007/s10142-015-0452-1>.
- Ali, N., Datta, S. K., & Datta, K., (2010). RNA interference in designing transgenic crops. *GM Crops*, 1(4), 207–213. <http://dx.doi.org/10.4161/gmcr.1.4.13344>.
- Allen, E., Xie, Z., Gustafson, A. M., Sung, G. H., Spatafora, J. W., & Carrington, J. C., (2004). Evolution of microRNA genes by inverted duplication of target gene sequences in *Arabidopsis thaliana*. *Nature Genetics*, 36(12), 1282–1290. <https://doi.org/10.1038/ng1478>.
- Ambros, V., Bartel, B., Bartel, D. P., Burge, C. B., Carrington, J. C., Chen, X., Dreyfuss, G., et al., (2003). A uniform system for microRNA annotation. *RNA*, 9(3), 277–279. <https://doi.org/10.1261/rna.2183803>.
- Amor, B. B., Wirth, S., Merchan, F., Laporte, P., D’aubenton-Carafa, Y., Hirsch, J., Maizel, A., et al., (2009). Novel long non-protein coding RNAs involved in *Arabidopsis* differentiation and stress responses. *Genome Research*, 19(1), 57–69. <https://doi.org/10.1101/gr.080275.108>.
- Aravin, A. A., Hannon, G. J., & Brennecke, J., (2007). The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. *Science*, 318(5851), 761–764. <https://doi.org/10.1126/science.1146484>.
- Archak, S., & Nagaraju, J., (2007). Computational prediction of rice (*Oryza sativa*) miRNA targets. *Genomics, Proteomics & Bioinformatics*, 5(3, 4), 196–206. [https://doi.org/10.1016/s1672-0229\(08\)60007-8](https://doi.org/10.1016/s1672-0229(08)60007-8).
- Arenas-Huerta, C., Perez, B., Rabanal, F., Blanco-Melo, D., De La Rosa, C., Estrada-Navarrete, G., Sanchez, F., et al., (2009). Conserved and novel miRNAs in the legume *Phaseolus vulgaris* in response to stress. *Plant Molecular Biology*, 70(4), 385–401. <https://doi.org/10.1007/s11103-009-9480-3>.
- Aukerman, M. J., & Sakai, H., (2003). Regulation of flowering time and floral organ identity by a microRNA and its APETALA2-like target genes. *The Plant Cell*, 15(11), 2730–2741. <https://doi.org/10.1105/tpc.016238>.
- Axtell, M. J., (2013). Classification and comparison of small RNAs from plants. *Annual Review of Plant Biology*, 64, 137–159. <https://doi.org/10.1146/annurev-arplant-050312-120043>.
- Axtell, M. J., Snyder, J. A., & Bartel, D. P., (2007). Common functions for diverse small RNAs of land plants. *The Plant Cell*, 19(6), 1750–1769. <https://doi.org/10.1105/tpc.107.051706>.

- Baldrich, P., Rutter, B. D., Karimi, H. Z., Podicheti, R., Meyers, B. C., & Innes, R. W., (2019). Plant extracellular vesicles contain diverse small RNA species and are enriched in 10-to 17-nucleotide “tiny” RNAs. *The Plant Cell*, 31(2), 315–324. <https://doi.org/10.1105/tpc.18.00872>.
- Bari, R., Pant, B. D., Stitt, M., & Scheible, W. R., (2006). PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiology*, 141(3), 988–999. <https://doi.org/10.1104/pp.106.079707>.
- Barrera-Figueroa, B. E., Gao, L., Wu, Z., Zhou, X., Zhu, J., Jin, H., et al., (2012). High throughput sequencing reveals novel and abiotic stress-regulated microRNAs in the inflorescences of rice. *BMC Plant Biology*, 12(1), 1–11. <http://dx.doi.org/10.1186/1471-2229-11-127>.
- Bartel, B., & Bartel, D. P., (2003). microRNAs: At the root of plant development? *Plant Physiology*, 132(2), 709–717. <https://doi.org/10.1104/pp.103.023630>.
- Bartel, D. P., (2004). microRNAs: Genomics, biogenesis, mechanism, and function. *Cell*, 116(2), 281–297. [https://doi.org/10.1016/s0092-8674\(04\)00045-5](https://doi.org/10.1016/s0092-8674(04)00045-5).
- Bartels, D., & Sunkar, R., (2005). Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences*, 24(1), 23–58. <http://dx.doi.org/10.1080/07352680590910410>.
- Baulcombe, D., (2002). Viral suppression of systemic silencing. *Trends in Microbiology*, 10(7), 306–308. [https://doi.org/10.1016/S0966-842X\(02\)02387-9](https://doi.org/10.1016/S0966-842X(02)02387-9).
- Baulcombe, D., (2004). RNA silencing in plants. *Nature*, 431(7006), 356–363. <https://doi.org/10.1038/nature02874>.
- Baulcombe, D., (2005). RNA silencing. *Trends in Biochemical Sciences*, 30(6), 290–293. <https://doi.org/10.1016/j.tibs.2005.04.012>.
- Bologna, N. G., & Voinnet, O., (2014). The diversity, biogenesis, and activities of endogenous silencing small RNAs in *Arabidopsis*. *Annual Review of Plant Biology*, 65, 473–503. <https://doi.org/10.1146/annurev-arplant-050213-035728>.
- Bonnet, E., He, Y., Billiau, K., & Van De, P. Y., (2010). TAPIR, a web server for the prediction of plant microRNA targets, including target mimics. *Bioinformatics*, 26(12), 1566–1568. <https://doi.org/10.1093/bioinformatics/btq233>.
- Borges, F., & Martienssen, R. A., (2015). The expanding world of small RNAs in plants. *Nature Reviews Molecular Cell Biology*, 16(12), 727–741. <https://doi.org/10.1038/nrm4085>.
- Borsani, O., Zhu, J., Verslues, P. E., Sunkar, R., & Zhu, J. K., (2005). Endogenous siRNAs derived from a pair of natural cis-antisense transcripts regulate salt tolerance in *Arabidopsis*. *Cell*, 123(7), 1279–1291. <https://doi.org/10.1016/j.cell.2005.11.035>.
- Bottino, M. C., Rosario, S., Grativol, C., Thiebaut, F., Rojas, C. A., Farrineli, L., Hemerly, A. S., & Ferreira, P. C. G., (2013). High-throughput sequencing of small RNA transcriptome reveals salt stress regulated microRNAs in sugarcane. *PLoS One*, 8(3), e59423. <https://doi.org/10.1371/journal.pone.0059423>.
- Boyko, A., Blevins, T., Yao, Y., Golubov, A., Bilichak, A., Ilnytskyy, Y., Hollander, J., Meins, Jr. F., & Kovalchuk, I., (2010). Transgenerational adaptation of *Arabidopsis* to stress requires DNA methylation and the function of dicer-like proteins. *PLoS One*, 5(3), e9514. <https://doi.org/10.1371/journal.pone.0009514>.
- Budak, H., & Zhang, B., (2017). microRNAs in model and complex organisms. *Genomics*, 15, 523–531. <https://doi.org/10.1007/s10142-015-0451-2>.
- Cai, Y., Yu, X., Hu, S., & Yu, J., (2009). A brief review on the mechanisms of miRNA regulation. *Genomics, Proteomics & Bioinformatics*, 7(4), 147–154. [https://doi.org/10.1016/S1672-0229\(08\)60044-3](https://doi.org/10.1016/S1672-0229(08)60044-3).



- Catalanotto, C., Cogoni, C., & Zardo, G., (2016). MicroRNA in control of gene expression: An overview of nuclear functions. *International Journal of Molecular Sciences*, 17(10), 1712. <https://doi.org/10.3390/ijms17101712>.
- Chalk, A. M., & Sonnhammer, E. L., (2008). siRNA specificity searching incorporating mismatch tolerance data. *Bioinformatics*, 24(10), 1316–1317. <https://doi.org/10.1093/bioinformatics/btn121>.
- Chan, S. W. L., Zhang, X., Bernatavichute, Y. V., & Jacobsen, S. E., (2006). Two-step recruitment of RNA-directed DNA methylation to tandem repeats. *PLoS Biol.*, 4(11), e363. <https://doi.org/10.1371/journal.pbio.0040363>.
- Chapman, E. J., & Carrington, J. C., (2007). Specialization and evolution of endogenous small RNA pathways. *Nature Reviews Genetics*, 8(11), 884–896. <https://doi.org/10.1038/nrg2179>.
- Chen, C., Zeng, Z., Liu, Z., & Xia, R., (2018). Small RNAs, emerging regulators critical for the development of horticultural traits. *Horticulture Research*, 5(1), 1–14. <https://doi.org/10.1038/s41438-018-0072-8>.
- Chen, L., Wang, T., Zhao, M., Tian, Q., & Zhang, W. H., (2012). Identification of aluminum-responsive microRNAs in *Medicago truncatula* by genome-wide high-throughput sequencing. *Planta.*, 235(2), 375–386. <https://doi.org/10.1007/s00425-011-1514-9>.
- Chen, Y. C. A., & Aravin, A. A., (2015). Non-coding RNAs in transcriptional regulation. *Current Molecular Biology Reports*, 1(1), 10–18. <https://doi.org/10.1007/s40610-015-0002-6>.
- Chinnusamy, V., Zhu, J., Zhou, T., & Zhu, J. K., (2007). Small RNAs: Big role in abiotic stress tolerance of plants. In: Jenks, M. A., Hasegawa, P. M., & Jain, S. M., (eds.), *Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops* (pp. 223–260). Springer: Dordrecht. [https://doi.org/10.1007/978-1-4020-5578-2\\_10](https://doi.org/10.1007/978-1-4020-5578-2_10).
- Choudhuri, S., (2009). Lesser known relatives of miRNA. *Biochem. Biophys. Res. Commun.*, 388(2), 177–180. <https://doi.org/10.1016/j.bbrc.2009.08.039>.
- Chowdhury, P., (2018). MicroRNA (miRNA) and small interfering RNA (siRNA): Biogenesis and functions in plants. In: Vats, S., (ed.), *Biotic and Abiotic Stress Tolerance in Plants* (pp. 323–348). Springer: Singapore. [https://doi.org/10.1007/978-981-10-9029-5\\_12](https://doi.org/10.1007/978-981-10-9029-5_12).
- Covarrubias, A. A., & Reyes, J. L., (2010). Post-transcriptional gene regulation of salinity and drought responses by plant microRNAs. *Plant, Cell & Environment*, 33(4), 481–489. <https://doi.org/10.1111/j.1365-3040.2009.02048.x>.
- Cui, W., Ning, J., Naik, U. P., & Duncan, M. K., (2004). OptiRNAi, an RNAi design tool. *Computer Methods and Programs in Biomedicine*, 75(1), 67–73. <https://doi.org/10.1016/j.cmpb.2003.09.002>.
- Cuperus, J. T., Fahlgren, N., & Carrington, J. C., (2011). Evolution and functional diversification of MIRNA genes. *The Plant Cell*, 23(2), 431–442. <https://doi.org/10.1105/tpc.110.082784>.
- Dalakouras, A., & Wassenegger, M., (2013). Revisiting RNA-directed DNA methylation. *RNA Biology*, 10(3), 453–455. <https://doi.org/10.4161/rna.23542>.
- De Fougerolles, A., Vornlocher, H. P., Maraganore, J., & Lieberman, J., (2007). Interfering with disease: A progress report on siRNA-based therapeutics. *Nature Reviews Drug Discovery*, 6(6), 443–453. <https://doi.org/10.1038/nrd2310>.
- Debernardi, J. M., Rodriguez, R. E., Mecchia, M. A., & Palatnik, J. F., (2012). Functional specialization of the plant miR396 regulatory network through distinct microRNA–target interactions. *PLoS Genet.*, 8(1), e1002419. <https://doi.org/10.1371/journal.pgen.1002419>.

- Deinlein, U., Stephan, A. B., Horie, T., Luo, W., Xu, G., & Schroeder, J. I., (2014). Plant salt-tolerance mechanisms. *Trends in Plant Science*, 19(6), 371–379. <https://doi.org/10.1016/j.tplants.2014.02.001>.
- Deng, P., Wang, L., Cui, L., Feng, K., Liu, F., Du, X., Tong, W., et al., (2015). Global identification of microRNAs and their targets in barley under salinity stress. *PLoS One*, 10(9), e0137990. <https://doi.org/10.1371/journal.pone.0137990>.
- Deuschle, K., Funck, D., Forlani, G., Stransky, H., Biehl, A., Leister, D., Van, D. G. E., et al., (2004). The role of  $\Delta 1$ -pyrroline-5-carboxylate dehydrogenase in proline degradation. *The Plant Cell*, 16(12), 3413–3425.
- Devi, S. R., Madhav, M., Kumar, G. R., Goel, A., Umakanth, B., Jahnavi, B., & Viraktamath, B., (2013). Identification of abiotic stress miRNA transcription factor binding motifs (TFBMs) in rice. *Gene*, 531(1), 15–22. <https://doi.org/10.1016/j.gene.2013.08.060>.
- Devkar, V., Thirumalaikumar, V. P., Xue, G. P., Vallarino, J. G., Tureckova, V., Strnad, M., Fernie, A. R., et al., (2020). Multifaceted regulatory function of tomato SITAFL1 in the response to salinity stress. *New Phytologist*, 225(4), 1681–1698. <https://doi.org/10.1111/nph.16247>.
- Dezulan, T., Remmert, M., Palatnik, J. F., Weigel, D., & Huson, D. H., (2006). Identification of plant microRNA homologs. *Bioinformatics*, 22(3), 359–360. <https://doi.org/10.1093/bioinformatics/bti802>.
- Ding, D., Zhang, L., Wang, H., Liu, Z., Zhang, Z., & Zheng, Y., (2009). Differential expression of miRNAs in response to salt stress in maize roots. *Annals of Botany*, 103(1), 29–38. <https://doi.org/10.1093/aob/mcn205>.
- Ding, Y., Chan, C. Y., & Lawrence, C. E., (2004). Sfold web server for statistical folding and rational design of nucleic acids. *Nucleic Acids Research*, 32(suppl\_2), W135–W141. <https://doi.org/10.1093/nar/gkh449>.
- Dolata, J., Bajczyk, M., Bielewicz, D., Niedojadlo, K., Niedojadlo, J., Pietrykowska, H., Walczak, W., et al., (2016). Salt stress reveals a new role for ARGONAUTE1 in miRNA biogenesis at the transcriptional and posttranscriptional levels. *Plant Physiology*, 172(1), 297–312. <https://doi.org/10.1104/pp.16.00830>.
- Dunoyer, P., Melnyk, C., Molnar, A., & Slotkin, R. K., (2013). Plant mobile small RNAs. *Cold Spring Harbor Perspectives in Biology*, 5(7), a017897. <https://doi.org/10.1101/cshperspect.a017897>.
- Dutta, T., Neelapu, N. R. R., Wani, S. H., & Surekha, C., (2020). Salt stress tolerance and small RNA. In: Guleria, P., & Kumar, V., (eds.), *Plant Small RNA* (pp. 191–207). Academic Press. <https://doi.org/10.1016/B978-0-12-817112-7.00010-9>.
- Eamens, A. L., Smith, N. A., Curtin, S. J., Wang, M. B., & Waterhouse, P. M., (2009). The *Arabidopsis thaliana* double-stranded RNA binding protein DRB1 directs guide strand selection from microRNA duplexes. *RNA*, 15(12), 2219–2235. <https://doi.org/10.1261/rna.1646909>.
- Fahlgren, N., Howell, M. D., Kasschau, K. D., Chapman, E. J., Sullivan, C. M., Cumbie, J. S., Givan, S. A., et al., (2007). High-throughput sequencing of *Arabidopsis* microRNAs: Evidence for frequent birth and death of MIRNA genes. *PLoS One*, 2(2), e219. <https://doi.org/10.1371/journal.pone.0000219>.
- Fang, Y., Xie, K., & Xiong, L., (2014). Conserved miR164-targeted NAC genes negatively regulate drought resistance in rice. *Journal of Experimental Botany*, 65(8), 2119–2135. <https://doi.org/10.1093/jxb/eru072>.

- Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C., (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, 391(6669), 806–811. <https://doi.org/10.1038/35888>.
- Floyd, S. K., & Bowman, J. L., (2004). Ancient microRNA target sequences in plants. *Nature*, 428(6982), 485–486. <https://doi.org/10.1038/428485a>.
- Frazier, T. P., Sun, G., Burklew, C. E., & Zhang, B., (2011). Salt and drought stresses induce the aberrant expression of microRNA genes in tobacco. *Molecular Biotechnology*, 49(2), 159–165. <https://doi.org/10.1007/s12033-011-9387-5>.
- Fu, R., Zhang, M., Zhao, Y., He, X., Ding, C., Wang, S., Feng, Y., et al., (2017). Identification of salt tolerance-related microRNAs and their targets in maize (*Zea mays* L.) using high-throughput sequencing and degradome analysis. *Frontiers in Plant Science*, 8, 864. <https://doi.org/10.3389/fpls.2017.00864>.
- Furini, A., Koncz, C., Salamini, F., & Bartels, D., (1997). High level transcription of a member of a repeated gene family confers dehydration tolerance to callus tissue of *Craterostigma plantagineum*. *The EMBO Journal*, 16(12), 3599–3608. <https://doi.org/10.1093/emboj/16.12.3599>.
- Gandikota, M., Birkenbihl, R. P., Höhmann, S., Cardon, G. H., Saedler, H., & Huijser, P., (2007). The miRNA156/157 recognition element in the 3' UTR of the *Arabidopsis* SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. *The Plant Journal*, 49(4), 683–693. <https://doi.org/10.1111/j.1365-3113x.2006.02983.x>.
- Gao, P., Bai, X., Yang, L., Lv, D., Li, Y., Cai, H., Ji, W., et al., (2010). Over-expression of osa-MIR396c decreases salt and alkali stress tolerance. *Planta*, 231(5), 991–1001. <https://doi.org/10.1007/s12374-017-0093-0>.
- Gao, P., Bai, X., Yang, L., Lv, D., Pan, X., Li, Y., Cai, H., et al., (2011). osa-MIR393: A salinity-and alkaline stress-related microRNA gene. *Molecular Biology Reports*, 38(1), 237–242. <http://dx.doi.org/10.1007/s11033-010-0100-8>.
- Gao, S., Yang, L., Zeng, H. Q., Zhou, Z. S., Yang, Z. M., Li, H., Sun, D., et al., (2016). A cotton miRNA is involved in regulation of plant response to salt stress. *Scientific Reports*, 6(1), 1–14. <https://doi.org/10.1038/srep19736>.
- Gehan, M. A., Greenham, K., Mockler, T. C., & McClung, C. R., (2015). Transcriptional networks—Crops, clocks, and abiotic stress. *Current Opinion in Plant Biology*, 24, 39–46. <https://doi.org/10.1016/j.pbi.2015.01.004>.
- Gentile, A., Dias, L. I., Mattos, R. S., Ferreira, T. H., & Menossi, M., (2015). microRNAs and drought responses in sugarcane. *Frontiers in Plant Science*, 6, 58. <https://doi.org/10.3389/fpls.2015.00058>.
- Gollmack, D., Lüking, I., & Yang, O., (2011). Plant tolerance to drought and salinity: Stress regulating transcription factors and their functional significance in the cellular transcriptional network. *Plant Cell Reports*, 30(8), 1383–1391. <https://doi.org/10.1007/s00299-011-1068-0>.
- Gong, W., Ren, Y., Zhou, H., Wang, Y., Kang, S., & Li, T., (2008). siDRM: An effective and generally applicable online siRNA design tool. *Bioinformatics*, 24(20), 2405–2406. <https://doi.org/10.1093/bioinformatics/btn442>.
- Griffiths-Jones, S., Grocock, R. J., Van, D. S., Bateman, A., & Enright, A. J., (2006). miRBase: MicroRNA sequences, targets and gene nomenclature. *Nucleic Acids Research*, 34(suppl\_1), D140–D144. <https://doi.org/10.1093/nar/gkj112>.
- Grobhans, H., & Filipowicz, W., (2008). The expanding world of small RNAs. *Nature*, 451(7177), 414–416. <https://doi.org/10.1371/journal.pgen.0040022>.

- Guan, D. G., Liao, J. Y., Qu, Z. H., Zhang, Y., & Qu, L. H., (2011). mirExplorer: Detecting microRNAs from genome and next generation sequencing data using the AdaBoost method with transition probability matrix and combined features. *RNA Biology*, 8(5), 922–934. <https://doi.org/10.4161/rna.8.5.16026>.
- Guleria, P., Mahajan, M., Bhardwaj, J., & Yadav, S. K., (2011). Plant small RNAs: Biogenesis, mode of action and their roles in abiotic stresses. *Genomics, Proteomics & Bioinformatics*, 9(6), 183–199.
- Guo, Q., Liu, Q., Smith, N. A., Liang, G., & Wang, M. B., (2016). RNA silencing in plants: Mechanisms, technologies and applications in horticultural crops. *Current Genomics*, 17(6), 476–489. <https://doi.org/10.2174/1389202917666160520103117>.
- Gupta, O. P., Meena, N. L., Sharma, I., & Sharma, P., (2014). Differential regulation of microRNAs in response to osmotic, salt and cold stresses in wheat. *Molecular Biology Reports*, 41(7), 4623–4629. <https://doi.org/10.1007/s11033-014-3333-0>.
- Gustafson, A. M., Allen, E., Givan, S., Smith, D., Carrington, J. C., & Kasschau, K. D., (2005). ASRP: The *Arabidopsis* small RNA project database. *Nucleic Acids Research*, 33(suppl\_1), D637–D640. <https://doi.org/10.1093/nar/gki127>.
- Gutierrez, L., Bussell, J. D., Păcurar, D. I., Schwambach, J., Păcurar, M., & Bellini, C., (2009). Phenotypic plasticity of adventitious rooting in *Arabidopsis* is controlled by complex regulation of AUXIN RESPONSE FACTOR transcripts and microRNA abundance. *The Plant Cell*, 21(10), 3119–3132. <https://doi.org/10.1105/tpc.108.064758>.
- Hackenberg, M., Gustafson, P., Langridge, P., & Shi, B. J., (2015). Differential expression of micro RNAs and other small RNAs in barley between water and drought conditions. *Plant Biotechnology Journal*, 13(1), 2–13. <https://doi.org/10.1093/dnares/dss037>.
- Hamilton, A., Voinnet, O., Chappell, L., & Baulcombe, D., (2002). Two classes of short interfering RNA in RNA silencing. *The EMBO Journal*, 21(17), 4671–4679. <https://doi.org/10.1093/emboj/cdf464>.
- Hasegawa, P. M., (2013). Sodium (Na<sup>+</sup>) homeostasis and salt tolerance of plants. *Environmental and Experimental Botany*, 92, 19–31. <https://doi.org/10.1016/j.envexpbot.2013.03.001>.
- Hasegawa, P. M., Bressan, R. A., Zhu, J. K., & Bohnert, H. J., (2000). Plant cellular and molecular responses to high salinity. *Annual Review of Plant Biology*, 51(1), 463–499. <https://doi.org/10.1146/annurev.arplant.51.1.463>.
- He, J., Jiang, Z., Gao, L., You, C., Ma, X., Wang, X., Xu, X., et al., (2019). Genome-wide transcript and small RNA profiling reveals transcriptomic responses to heat stress. *Plant Physiology*, 181(2), 609–629. <https://doi.org/10.1104/pp.19.00403>.
- Henschel, A., Buchholz, F., & Habermann, B., (2004). DEQOR: A web-based tool for the design and quality control of siRNAs. *Nucleic Acids Research*, 32(suppl\_2), W113–W120. <https://doi.org/10.1093/nar/gkh408>.
- Hinas, A., Wright, A. J., & Hunter, C. P., (2012). SID-5 is an endosome-associated protein required for efficient systemic RNAi in *C. elegans*. *Current Biology*, 22(20), 1938–1943. <https://doi.org/10.1016/j.cub.2012.08.020>.
- Hirayama, T., & Shinozaki, K., (2010). Research on plant abiotic stress responses in the post-genome era: Past, present and future. *The Plant Journal*, 61(6), 1041–1052. <https://doi.org/10.1111/j.1365-313X.2010.04124.x>.
- Horn, T., Sandmann, T., & Boutros, M., (2010). Design and evaluation of genome-wide libraries for RNA interference screens. *Genome Biology*, 11(6), 1–12. <https://doi.org/10.1186/gb-2010-11-6-r61>.

- Huang, T. H., Fan, B., Rothschild, M. F., Hu, Z. L., Li, K., & Zhao, S. H., (2007). MiRFinder: An improved approach and software implementation for genome-wide fast microRNA precursor scans. *BMC Bioinformatics*, 8(1), 1–10. <https://doi.org/10.1186/1471-2105-8-341>.
- Hunter, C., Winston, W., Molodowitch, C., Feinberg, E., Shih, J., Sutherlin, M., Wright, A., & Fitzgerald, M., (2006). Systemic RNAi in *Caenorhabditis elegans*. In: *Cold Spring Harbor Symposia on Quantitative Biology* (Vol. 71, pp. 95–100). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA. <https://doi.org/10.1101/sqb.2006.71.060>.
- Huntzinger, E., & Izaurralde, E., (2011). Gene silencing by microRNAs: Contributions of translational repression and mRNA decay. *Nature Reviews Genetics*, 12(2), 99–110. <https://doi.org/10.1038/nrg2936>.
- Jagadeeswaran, G., Saini, A., & Sunkar, R., (2009). Biotic and abiotic stress down-regulate miR398 expression in *Arabidopsis*. *Planta*, 229(4), 1009–1014. <https://doi.org/10.1007/s00425-009-0889-3>.
- Johnson, W. L., & Straight, A. F., (2017). RNA-mediated regulation of heterochromatin. *Current Opinion in Cell Biology*, 46, 102–109. <https://doi.org/10.1016/j.ceb.2017.05.004>.
- Jones-Rhoades, M. W., Bartel, D. P., & Bartel, B., (2006). microRNAs and their regulatory roles in plants. *Annu. Rev. Plant Biol.*, 57, 19–53. <https://doi.org/10.1146/annurev.arplant.57.032905.105218>.
- Jorgensen, R. A., Atkinson, R. G., Forster, R. L., & Lucas, W. J., (1998). An RNA-based information superhighway in plants. *Science*, 279(5356), 1486–1487. <https://doi.org/10.1126/science.279.5356.1486>.
- Joshi, R., Gupta, P., Singla-Pareek, S. L., & Pareek, A., (2017). Biomass production and salinity response in plants: Role of microRNAs. *Indian Journal of Plant Physiology*, 22(4), 448–457. <https://doi.org/10.1007/s40502-017-0327-7>.
- Joshi, R., Wani, S. H., Singh, B., Bohra, A., Dar, Z. A., Lone, A. A., Pareek, A., & Singla-Pareek, S. L., (2016). Transcription factors and plants response to drought stress: Current understanding and future directions. *Frontiers in Plant Science*, 7, 1029. <https://doi.org/10.3389/fpls.2016.01029>.
- Jover-Gil, S., Candela, H., & Ponce, M. R., (2004). Plant microRNAs and development. *International Journal of Developmental Biology*, 49(5, 6), 733–744. <https://doi.org/10.1093/aob/mcu132>.
- Kalantidis, K., Schumacher, H. T., Alexiadis, T., & Helm, J. M., (2008). RNA silencing movement in plants. *Biology of the Cell*, 100(1), 13–26. <https://doi.org/10.1042/BC20070079>.
- Kamthan, A., Chaudhuri, A., Kamthan, M., & Datta, A., (2015). Small RNAs in plants: Recent development and application for crop improvement. *Frontiers in Plant Science*, 6, 208. <https://doi.org/10.3389/fpls.2015.00208>.
- Kawaji, H., & Hayashizaki, Y., (2008). Exploration of small RNAs. *PLoS Genet.*, 4(1), e22.
- Khan, Y., Yadav, A., Bonthala, V. S., Muthamilarasan, M., Yadav, C. B., & Prasad, M., (2014). Comprehensive genome-wide identification and expression profiling of foxtail millet [*Setaria italica* (L.)] miRNAs in response to abiotic stress and development of miRNA database. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 118(2), 279–292. <https://doi.org/10.1007/s11240-014-0480-x>.
- Khare, T., Kumar, V., & Kishor, P. K., (2015). Na<sup>+</sup> and Cl<sup>−</sup> ions show additive effects under NaCl stress on induction of oxidative stress and the responsive antioxidative defense in rice. *Protoplasma*, 252(4), 1149–1165. <https://doi.org/10.1007/s00709-014-0749-2>.

- Khare, T., Shriram, V., & Kumar, V., (2018). RNAi technology: The role in development of abiotic stress-tolerant crops. In: Wani, S. H., (ed.), *Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants* (pp. 117–133). Academic Press. <https://doi.org/10.1016/B978-0-12-813066-7.00008-5>.
- Khraiwesh, B., Zhu, J. K., & Zhu, J., (2012). Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, 1819(2), 137–148. <https://doi.org/10.1016/j.bbagr.2011.05.001>.
- Kim, S. K., Nam, J. W., Rhee, J. K., Lee, W. J., & Zhang, B. T., (2006). miTarget: MicroRNA target gene prediction using a support vector machine. *BMC Bioinformatics*, 7(1), 1–12. <https://doi.org/10.1186/1471-2105-7-411>.
- Kim, V. N., (2005). MicroRNA biogenesis: Coordinated cropping and dicing. *Nature Reviews Molecular Cell Biology*, 6(5), 376–385. <https://doi.org/10.1038/nrm1644>.
- Kim, V. N., (2005). Small RNAs: Classification, biogenesis, and function. *Molecules & Cells*, 19(1), 1–15.
- Kim, V. N., Han, J., & Siomi, M. C., (2009). Biogenesis of small RNAs in animals. *Nature Reviews Molecular Cell Biology*, 10(2), 126–139. <https://doi.org/10.1038/nrm2632>.
- Kinoshita, T., & Seki, M., (2014). Epigenetic memory for stress response and adaptation in plants. *Plant and Cell Physiology*, 55(11), 1859–1863. <https://doi.org/10.1093/pcp/pcu125>.
- Kong, Y., Elling, A. A., Chen, B., & Deng, X., (2010). Differential expression of microRNAs in maize inbred and hybrid lines during salt and drought stress. *American Journal of Plant Sciences*, 1(02), 69. <https://doi.org/10.4236/ajps.2010.12009>.
- Kooter, J. M., Matzke, M. A., & Meyer, P., (1999). Listening to the silent genes: Transgene silencing, gene regulation and pathogen control. *Trends in Plant Science*, 4(9), 340–347. [https://doi.org/10.1016/S1360-1385\(99\)01467-3](https://doi.org/10.1016/S1360-1385(99)01467-3).
- Koroban, N., Kudryavtseva, A., Krasnov, G., Sadritdinova, A., Fedorova, M., Snezhkina, A., Bolsheva, N., et al., (2016). The role of microRNA in abiotic stress response in plants. *Molecular Biology*, 50(3), 337–343. <https://doi.org/10.1134/S0026893316020102>.
- Ku, Y. S., Wong, J. W. H., Mui, Z., Liu, X., Hui, J. H. L., Chan, T. F., & Lam, H. M., (2015). Small RNAs in plant responses to abiotic stresses: Regulatory roles and study methods. *International Journal of Molecular Sciences*, 16(10), 24532–24554. <https://doi.org/10.3390/ijms161024532>.
- Kumar, R., (2014). Role of microRNAs in biotic and abiotic stress responses in crop plants. *Applied Biochemistry and Biotechnology*, 174(1), 93–115. <https://doi.org/10.1007/s12010-014-0914-2>.
- Kumar, V., Khare, T., Shriram, V., & Wani, S. H., (2018). Plant small RNAs: The essential epigenetic regulators of gene expression for salt-stress responses and tolerance. *Plant Cell Reports*, 37(1), 61–75. <https://doi.org/10.1007/s00299-017-2210-4>.
- Kurihara, Y., & Watanabe, Y., (2004). *Arabidopsis* micro-RNA biogenesis through dicer-like 1 protein functions. *Proceedings of the National Academy of Sciences*, 101(34), 12753–12758. <https://doi.org/10.1073/pnas.0403115101>.
- Kurihara, Y., Takashi, Y., & Watanabe, Y., (2006). The interaction between DCL1 and HYL1 is important for efficient and precise processing of pri-miRNA in plant microRNA biogenesis. *RNA*, 12(2), 206–212. <https://doi.org/10.1261/rna.2146906>.
- Ladunga, I., (2007). More complete gene silencing by fewer siRNAs: Transparent optimized design and biophysical signature. *Nucleic Acids Research*, 35(2), 433–440. <https://doi.org/10.1093/nar/gkl1065>.

- Lagos-Quintana, M., Rauhut, R., Lendeckel, W., & Tuschl, T., (2001). Identification of novel genes coding for small expressed RNAs. *Science*, 294(5543), 853–858. <https://doi.org/10.1126/science.1064921>.
- Lee, Y., Kim, M., Han, J., Yeom, K. H., Lee, S., Baek, S. H., & Kim, V. N., (2004). MicroRNA genes are transcribed by RNA polymerase II. *The EMBO Journal*, 23(20), 4051–4060. <https://doi.org/10.1038/sj.emboj.7600385>.
- Lelandais-Brière, C., Naya, L., Sallet, E., Calenge, F., Frugier, F., Hartmann, C., Gouzy, J., & Crespi, M., (2009). Genome-wide *Medicago truncatula* small RNA analysis revealed novel microRNAs and isoforms differentially regulated in roots and nodules. *The Plant Cell*, 21(9), 2780–2796. <https://doi.org/10.1007/s10265-015-0715-x>.
- Leung, A. K., & Sharp, P. A., (2010). MicroRNA functions in stress responses. *Molecular Cell*, 40(2), 205–215. <https://doi.org/10.1016/j.molcel.2010.09.027>.
- Li, B., Duan, H., Li, J., Deng, X. W., Yin, W., & Xia, X., (2013). Global identification of miRNAs and targets in *Populus euphratica* under salt stress. *Plant Molecular Biology*, 81(6), 525–539. <https://doi.org/10.1007/s11103-013-0010-y>.
- Li, C., Li, Y., Bai, L., He, C., & Yu, X., (2016). Dynamic expression of miRNAs and their targets in the response to drought stress of grafted cucumber seedlings. *Horticultural Plant Journal*, 2(1), 41–49. <https://doi.org/10.1016/j.hpj.2016.02.002>.
- Li, C., Lv, J., Zhao, X., Ai, X., Zhu, X., Wang, M., Zhao, S., & Xia, G., (2010). TaCHP: A wheat zinc finger protein gene down-regulated by abscisic acid and salinity stress plays a positive role in stress tolerance. *Plant Physiology*, 154(1), 211–221. <https://doi.org/10.1104/pp.110.161182>.
- Li, F., Ma, F., Ji, X., Wang, S., Sun, Y., Yu, L., Li, G., & Han, Z., (2012). Methods for identifying small RNA genes. *Advances in Insect Physiology*, 42, 155–194. <https://doi.org/10.1016/B978-0-12-387680-5.00005-7>.
- Li, G., Tai, F. J., Zheng, Y., Luo, J., Gong, S. Y., Zhang, Z. T., & Li, X. B., (2010). Two cotton Cys2/His2-type zinc-finger proteins, GhDi19-1 and GhDi19-2, are involved in plant response to salt/drought stress and abscisic acid signaling. *Plant Molecular Biology*, 74(4, 5), 437–452. <https://doi.org/10.1007/s11103-010-9684-6>.
- Li, H., Dong, Y., Yin, H., Wang, N., Yang, J., Liu, X., Wang, Y., Wu, J., & Li, X., (2011). Characterization of the stress associated microRNAs in *Glycine max* by deep sequencing. *BMC Plant Biology*, 11(1), 1–12. <https://doi.org/10.1186/1471-2229-11-170>.
- Li, J., Guo, G., Guo, W., Guo, G., Tong, D., Ni, Z., Sun, Q., & Yao, Y., (2012). miRNA164-directed cleavage of ZmNAC1 confers lateral root development in maize (*Zea mays* L.). *BMC Plant Biology*, 12(1), 1–14. <https://doi.org/10.1186/1471-2229-12-220>.
- Li, Y. F., Zheng, Y., Addo-Quaye, C., Zhang, L., Saini, A., Jagadeeswaran, G., Axtell, M. J., et al., (2010). Transcriptome-wide identification of microRNA targets in rice. *The Plant Journal*, 62(5), 742–759. <https://doi.org/10.1111/j.1365-313X.2010.04187.x>.
- Li, Z., Xu, R., & Li, N., (2018). microRNAs from plants to animals, do they define a new messenger for communication? *Nutrition & Metabolism*, 15(1), 1–21. <https://doi.org/10.1186/s12986-018-0305-8>.
- Liu, H. H., Tian, X., Li, Y. J., Wu, C. A., & Zheng, C. C., (2008). Microarray-based analysis of stress-regulated microRNAs in *Arabidopsis thaliana*. *RNA*, 14(5), 836–843. <https://doi.org/10.1261/rna.895308>.
- Llave, C., Xie, Z., Kasschau, K. D., & Carrington, J. C., (2002). Cleavage of scarecrow-like mRNA targets directed by a class of *Arabidopsis* miRNA. *Science*, 297(5589), 2053–2056. <https://doi.org/10.1126/science.1076311>.

- Long, R., Zhang, F., Li, Z., Li, M., Cong, L., Kang, J., Zhang, T., Zhao, Z., Sun, Y., & Yang, Q., (2015). Isolation and functional characterization of salt-stress induced RCI2-like genes from *Medicago sativa* and *Medicago truncatula*. *J. Plant Res.*, 128(4), 697–707. <https://doi.org/10.1007/s10265-015-0715-x>.
- Lu, C., Tej, S. S., Luo, S., Haudenschild, C. D., Meyers, B. C., & Green, P. J., (2005). Elucidation of the small RNA component of the transcriptome. *Science*, 309(5740), 1567–1569. <https://doi.org/10.1126/science.1114112>.
- Lu, J., Fu, Y., Kumar, S., Shen, Y., Zeng, K., Xu, A., Carthew, R., & Wu, C. I., (2008). Adaptive evolution of newly emerged micro-RNA genes in *Drosophila*. *Molecular Biology and Evolution*, 25(5), 929–938. <https://doi.org/10.1093/molbev/msn040>.
- Lu, S., Sun, Y. H., & Chiang, V. L., (2008). Stress-responsive microRNAs in *Populus*. *The Plant Journal*, 55(1), 131–151. <https://doi.org/10.1111/j.1365-313X.2008.03497.x>.
- Lu, W., Li, J., Liu, F., Gu, J., Guo, C., Xu, L., Zhang, H., & Xiao, K., (2011). Expression pattern of wheat miRNAs under salinity stress and prediction of salt-inducible miRNAs targets. *Frontiers of Agriculture in China*, 5(4), 413–422. <https://doi.org/10.1007/s11703-011-1133-z>.
- Lu, Y., & Yang, X., (2010). Computational identification of novel microRNAs and their targets in *Vigna unguiculata*. *Comparative and Functional Genomics*, 2010, 128297. <https://doi.org/10.1155/2010/128297>.
- Lu, Z. J., & Mathews, D. H., (2008). Efficient siRNA selection using hybridization thermodynamics. *Nucleic Acids Research*, 36(2), 640–647. <https://doi.org/10.1093/nar/gkm920>.
- Luan, M., Xu, M., Lu, Y., Zhang, Q., Zhang, L., Zhang, C., Fan, Y., et al., (2014). Family-wide survey of miR169s and NF-YAs and their expression profiles response to abiotic stress in maize roots. *PloS One*, 9(3), e91369. <https://doi.org/10.1371/journal.pone.0091369>.
- Lv, S., Nie, X., Wang, L., Du, X., Biradar, S. S., Jia, X., & Weining, S., (2012). Identification and characterization of microRNAs from barley (*Hordeum vulgare* L.) by high-throughput sequencing. *International Journal of Molecular Sciences*, 13(3), 2973–2984. <https://doi.org/10.3390/ijms13032973>.
- Ma, C., Burd, S., & Lers, A., (2015). miR 408 is involved in abiotic stress responses in *Arabidopsis*. *The Plant Journal*, 84(1), 169–187. <https://doi.org/10.1111/tpj.12999>.
- Macovei, A., & Tuteja, N., (2012). microRNAs targeting DEAD-box helicases are involved in salinity stress response in rice (*Oryza sativa* L.). *BMC Plant Biology*, 12(1), 1–12. <https://doi.org/10.1186/1471-2229-12-183>.
- Mallory, A. C., & Vaucheret, H., (2006). Functions of microRNAs and related small RNAs in plants. *Nature Genetics*, 38(6), S31–S36. <https://doi.org/10.1038/ng1791>.
- Manavella, P. A., Yang, S. W., & Palatnik, J., (2019). Keep calm and carry on: MiRNA biogenesis under stress. *The Plant Journal*, 99(5), 832–843. <https://doi.org/10.1111/tpj.14369>.
- Marin-Gonzalez, E., & Suarez-Lopez, P., (2012). And yet it moves: Cell-to-cell and long-distance signaling by plant microRNAs. *Plant Science*, 196, 18–30. <https://doi.org/10.1016/j.plantsci.2012.07.009>.
- Martienssen, R., & Moazed, D., (2015). RNAi and heterochromatin assembly. *Cold Spring Harbor Perspectives in Biology*, 7(8), a019323. <https://doi.org/10.1101/cshperspect.a019323>.



- Mecchia, M. A., Debernardi, J. M., Rodriguez, R. E., Schommer, C., & Palatnik, J. F., (2013). MicroRNA miR396 and RDR6 synergistically regulate leaf development. *Mechanisms of Development*, 130(1), 2–13. <https://doi.org/10.1016/j.mod.2012.07.005>.
- Medina, C., Da Rocha, M., Magliano, M., Raptopoulou, A., Marteu, N., Lebrigand, K., Abad, P., et al., (2018). Characterization of siRNAs clusters in *Arabidopsis thaliana* galls induced by the root-knot nematode *Meloidogyne incognita*. *BMC Genomics*, 19(1), 1–16. <https://doi.org/10.1186/s12864-018-5296-3>.
- Mello, C. C., & Conte, D., (2004). Revealing the world of RNA interference. *Nature*, 431(7006), 338. <https://doi.org/10.1038/nature02872>.
- Meyer, S. U., Stoecker, K., Sass, S., Theis, F. J., & Pfaffl, M. W., (2014). Posttranscriptional regulatory networks: From expression profiling to integrative analysis of mRNA and microRNA data. In: Biassoni, R., & Raso, A., (eds.), *Quantitative Real-Time PCR* (pp. 165–188). Humana Press: New York, NY. [https://doi.org/10.1007/978-1-4939-0733-5\\_15](https://doi.org/10.1007/978-1-4939-0733-5_15).
- Mhuantong, W., & Wichadakul, D., (2009). MicroPC (μPC): A comprehensive resource for predicting and comparing plant microRNAs. *BMC Genomics*, 10(1), 1–8. <https://doi.org/10.1186/1471-2164-10-366>.
- Milev, I., Yahubyan, G., Minkov, I., & Baev, V., (2011). miRTour: Plant miRNA and target prediction tool. *Bioinformatics*, 6(6), 248. 10.6026/97320630006248.
- Mirlohi, S., & He, Y., (2016). Small RNAs in plant response to abiotic stress. In: Shanker, A., (ed.), *Biotic and Biotic Stress in Plants-Recent Advances and Future Perspectives* (pp. 63–80). Intech: Croatia. <http://dx.doi.org/10.5772/61834>.
- Mondal, T. K., Panda, A. K., Rawal, H. C., & Sharma, T. R., (2018). Discovery of microRNA-target modules of African rice (*Oryza glaberrima*) under salinity stress. *Scientific Reports*, 8(1), 1–11. <https://doi.org/10.1038/s41598-017-18206-z>.
- Montes, R. A. C., De Paoli, E., Accerbi, M., Rymarquis, L. A., Mahalingam, G., Marsch-Martínez, N., Meyers, B. C., et al., (2014). Sample sequencing of vascular plants demonstrates widespread conservation and divergence of microRNAs. *Nature Communications*, 5(1), 1–15. <https://doi.org/10.1038/ncomms4722>.
- Muchate, N. S., Nikalje, G. C., Rajurkar, N. S., Suprasanna, P., & Nikam, T. D., (2016). Plant salt stress: Adaptive responses, tolerance mechanism and bioengineering for salt tolerance. *The Botanical Review*, 82(4), 371–406. <https://doi.org/10.1007/s12229-016-9173-y>.
- Munns, R., & Tester, M., (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59, 651–681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>.
- Mutum, R. D., Balyan, S. C., Kansal, S., Agarwal, P., Kumar, S., Kumar, M., & Raghuvanshi, S., (2013). Evolution of variety-specific regulatory schema for expression of osa-miR408 in indica rice varieties under drought stress. *The FEBS Journal*, 280(7), 1717–1730. <https://doi.org/10.1038/s41598-017-15450-1>.
- Naito, Y., Yoshimura, J., Morishita, S., & Ui-Tei, K., (2009). siDirect 2.0: Updated software for designing functional siRNA with reduced seed-dependent off-target effect. *BMC Bioinformatics*, 10(1), 1–8. <https://doi.org/10.1186/1471-2105-10-392>.
- Nakano, M., Nobuta, K., Vemaraju, K., Tej, S. S., Skogen, J. W., & Meyers, B. C., (2006). Plant MPSS databases: Signature-based transcriptional resources for analyses of mRNA and small RNA. *Nucleic Acids Research*, 34(suppl\_1), D731–D735. <https://doi.org/10.1093/nar/gkj077>.
- Nakashima, K., Yamaguchi-Shinozaki, K., & Shinozaki, K., (2014). The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses

- including drought, cold, and heat. *Frontiers in Plant Science*, 5, 170. <https://doi.org/10.3389/fpls.2014.00170>.
- Napoli, C., Lemieux, C., & Jorgensen, R., (1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell*, 2(4), 279–289. <https://doi.org/10.1105/tpc.2.4.279>.
- Naqvi, A. R., Sarwat, M., Hasan, S., & Roychodhury, N., (2012). Biogenesis, functions and fate of plant microRNAs. *Journal of Cellular Physiology*, 227(9), 3163–3168. <https://doi.org/10.1002/jcp.24052>.
- Noman, A., & Aqeel, M., (2017). miRNA-based heavy metal homeostasis and plant growth. *Environmental Science and Pollution Research*, 24(11), 10068–10082. doi: 10.1007/s11356-017-8593-5.
- Nosaka, M., Itoh, J. I., Nagato, Y., Ono, A., Ishiwata, A., & Sato, Y., (2012). Role of transposon-derived small RNAs in the interplay between genomes and parasitic DNA in rice. *PLoS Genet.*, 8(9), e1002953. <https://doi.org/10.1371/journal.pgen.1002953>.
- Ossowski, S., Schwab, R., & Weigel, D., (2008). Gene silencing in plants using artificial microRNAs and other small RNAs. *The Plant Journal*, 53(4), 674–690. <https://doi.org/10.1111/j.1365-3113x.2007.03328.x>.
- Pandey, R., Joshi, G., Bhardwaj, A. R., Agarwal, M., & Katiyar-Agarwal, S., (2014). A comprehensive genome-wide study on tissue-specific and abiotic stress-specific miRNAs in *Triticum aestivum*. *PLoS One*, 9(4), e95800. <https://doi.org/10.1371/journal.pone.0095800>.
- Park, J. H., & Shin, C., (2014). MicroRNA-directed cleavage of targets: Mechanism and experimental approaches. *BMB Reports*, 47(8), 417. <https://doi.org/10.5483/bmbrep.2014.47.8.109>.
- Park, Y. K., Park, S. M., Choi, Y. C., Lee, D., Won, M., & Kim, Y. J., (2008). AsiDesigner: Exon-based siRNA design server considering alternative splicing. *Nucleic Acids Research*, 36(suppl 2), W97–W103. <https://doi.org/10.1093/nar/gkn280>.
- Parmar, S., Gharat, S. A., Tagirasa, R., Chandra, T., Behera, L., Dash, S. K., & Shaw, B. P., (2020). Identification and expression analysis of miRNAs and elucidation of their role in salt tolerance in rice varieties susceptible and tolerant to salinity. *PloS One*, 15(4), e0230958. <https://doi.org/10.1371/journal.pone.0230958>.
- Parmer, S., & Shaw, B., (2018). Salt stress tolerance in plants: The role of miRNAs. *Adv Plants Agric. Res.*, 8(6), 411–415. <https://doi.org/10.15406/apar.2018.08.00360>.
- Paul, S., Kundu, A., & Pal, A., (2011). Identification and validation of conserved microRNAs along with their differential expression in roots of *Vigna unguiculata* grown under salt stress. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 105(2), 233–242. <https://doi.org/10.1007/s11240-010-9857-7>.
- Pegler, J. L., Oultram, J. M., Grof, C. P., & Eamens, A. L., (2019). Profiling the abiotic stress responsive microRNA landscape of *Arabidopsis thaliana*. *Plants*, 8(3), 58. <https://doi.org/10.3390/plants8030058>.
- Peters, L., & Meister, G., (2007). Argonaute proteins: Mediators of RNA silencing. *Molecular Cell*, 26(5), 611–623. <https://doi.org/10.1016/j.molcel.2007.05.001>.
- Phillips, J. R., Dalmay, T., & Bartels, D., (2007). The role of small RNAs in abiotic stress. *FEBS Letters*, 581(19), 3592–3597. <https://doi.org/10.1016/j.febslet.2007.04.007>.
- Pradhan, A., Naik, N., & Sahoo, K. K., (2015). RNAi mediated drought and salinity stress tolerance in plants. *American Journal of Plant Sciences*, 6(12), 1990. <http://dx.doi.org/10.4236/ajps.2015.612200>.

- Priest, H. D., Fox, S. E., Rowley, E. R., Murray, J. R., Michael, T. P., & Mockler, T. C., (2014). Analysis of global gene expression in *Brachypodium distachyon* reveals extensive network plasticity in response to abiotic stress. *PLoS One*, 9(1), e87499. <https://doi.org/10.1371/journal.pone.0087499>.
- Qiu, Q., Ma, T., Hu, Q., Liu, B., Wu, Y., Zhou, H., Wang, Q., et al., (2011). Genome-scale transcriptome analysis of the desert poplar, *Populus euphratica*. *Tree Physiology*, 31(4), 452–461. <https://doi.org/10.1093/treephys/tpr015>.
- Reinhart, B. J., Weinstein, E. G., Rhoades, M. W., Bartel, B., & Bartel, D. P., (2002). microRNAs in plants. *Genes & Development*, 16(13), 1616–1626. <https://doi.org/10.1101/gad.1004402>.
- Ritchie, W., Theodoule, F. X., & Gautheret, D., (2008). Mireval: A web tool for simple microRNA prediction in genome sequences. *Bioinformatics*, 24(11), 1394–1396. <https://doi.org/10.1093/bioinformatics/btn137>.
- Rusinov, V., Baev, V., Minkov, I. N., & Tabler, M., (2005). MicroInspector: A web tool for detection of miRNA binding sites in an RNA sequence. *Nucleic Acids Research*, 33(suppl\_2), W696–W700. <https://doi.org/10.1093/nar/gki364>.
- Sahu, P. P., Pandey, G., Sharma, N., Puranik, S., Muthamilarasan, M., & Prasad, M., (2013). Epigenetic mechanisms of plant stress responses and adaptation. *Plant Cell Reports*, 32(8), 1151–1159. <https://doi.org/10.1007/s00299-013-1462-x>.
- Sanan-Mishra, N., Jailani, A. A. K., Mandal, B., & Mukherjee, S. K., (2021). Secondary siRNAs in plants: Biosynthesis, various functions, and applications in virology. *Frontiers in Plant Science*, 12, 610283. <https://doi.org/10.3389/fpls.2021.610283>.
- Sethupathy, P., Corda, B., & Hatzigeorgiou, A. G., (2006). TarBase: A comprehensive database of experimentally supported animal microRNA targets. *RNA*, 12(2), 192–197. <https://doi.org/10.1261/rna.2239606>.
- Shah, J. K., Garner, H. R., White, M. A., Shames, D. S., & Minna, J. D., (2007). siR: siRNA information resource, a web-based tool for siRNA sequence design and analysis and an open access siRNA database. *BMC Bioinformatics*, 8, 178. <https://doi.org/10.1186/1471-2105-8-178>.
- Sharma, N., Tripathi, A., & Sanan-Mishra, N., (2015). Profiling the expression domains of a rice-specific microRNA under stress. *Frontiers in Plant Science*, 6, 333. <https://doi.org/10.3389/fpls.2015.00333>.
- Shukla, G. C., Singh, J., & Barik, S., (2011). microRNAs: Processing, maturation, target recognition and regulatory functions. *Molecular and Cellular Pharmacology*, 3(3), 83.
- Si, J., Zhou, T., Bo, W., Xu, F., & Wu, R., (2014). Genome-wide analysis of salt-responsive and novel microRNAs in *Populus euphratica* by deep sequencing. *BMC Genetics*, 15(S6), 1–11. <https://doi.org/10.1186/1471-2156-15-S1-S6>.
- Singh, A., Roy, S., Singh, S., Das, S. S., Gautam, V., Yadav, S., Kumar, A., Samantha, S., & Sarkar, A. K., (2017). Phytohormonal crosstalk modulates the expression of miR166/165s, target class III HD-ZIPs, and KANADI genes during root growth in *Arabidopsis thaliana*. *Sci Rep.*, 7(1), 3408. <https://doi.org/10.1038/s41598-017-03632-w>.
- Song, J. B., Gao, S., Sun, D., Li, H., Shu, X. X., & Yang, Z. M., (2013). miR394 and LCR are involved in *Arabidopsis* salt and drought stress responses in an abscisic acid-dependent manner. *BMC Plant Biology*, 13(1), 1–16. <https://doi.org/10.1186/1471-2229-13-210>.
- Sun, G., (2012). microRNAs and their diverse functions in plants. *Plant Molecular Biology*, 80(1), 17–36. <https://doi.org/10.1007/s11103-011-9817-6>.

- Sun, X., Korir, N. K., Han, J., Shangguan, L. F., Kayesh, E., Leng, X. P., & Fang, J. G., (2012). Characterization of grapevine microR164 and its target genes. *Molecular Biology Reports*, 39(10), 9463–9472. <https://doi.org/10.1007/s11033-012-1811-9>.
- Sun, X., Xu, L., Wang, Y., Yu, R., Zhu, X., Luo, X., Gong, Y., et al., (2015). Identification of novel and salt-responsive miRNAs to explore miRNA-mediated regulatory network of salt stress response in radish (*Raphanus sativus* L.). *BMC Genomics*, 16(1), 1–16. <https://doi.org/10.1038/srep14024>.
- Sunkar, R., & Jagadeeswaran, G., (2008). *In silico* identification of conserved microRNAs in large number of diverse plant species. *BMC Plant Biology*, 8(1), 1–13. <https://doi.org/10.1186/1471-2229-8-37>.
- Sunkar, R., & Zhu, J. K., (2004). Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. *The Plant Cell*, 16(8), 2001–2019. <https://doi.org/10.1105/tpc.104.022830>.
- Sunkar, R., Li, Y. F., & Jagadeeswaran, G., (2012). Functions of microRNAs in plant stress responses. *Trends in Plant Science*, 17(4), 196–203. <https://doi.org/10.1016/j.tplants.2012.01.010>.
- Surekha, C., Aruna, L., Hossain, M. A., Wani, S. H., & Neelapu, N. R. R., (2015). Present status and future prospects of transgenic approaches for salt tolerance in plants/crop plants. In: Wani, S. H., & Hossain, M. A., (eds.), *Managing Salt Tolerance in Plants: Molecular and Genomic Perspectives* (pp. 329–352). CRC Press: New York, NY, USA.
- Szczesniak, M. W., Deorowicz, S., Gapski, J., Kaczynski, L., & Makołowska, I., (2012). miRNEST database: An integrative approach in microRNA search and annotation. *Nucleic Acids Research*, 40(D1), D198–D204. <https://doi.org/10.1093/nar/gkr1159>.
- Tabara, H., Grishok, A., & Mello, C. C., (1998). RNAi in *C. elegans*: Soaking in the genome sequence. *Science*, 282(5388), 430, 431. <https://doi.org/10.1126/science.282.5388.430>.
- Thakur, A., (2003). RNA interference revolution. *Electronic Journal of Biotechnology*, 6(1), 1–2. <https://doi.org/10.1038/ncomms4722>.
- Thieme, C. J., Gramzow, L., Lobbes, D., & Theißen, G., (2011). SplamiR—prediction of spliced miRNAs in plants. *Bioinformatics*, 27(9), 1215–1223. [10.1186/1471-2164-10-366](https://doi.org/10.1186/1471-2164-10-366). <https://doi.org/10.1093/bioinformatics/btr132>.
- Vaucheret, H., (2006). Post-transcriptional small RNA pathways in plants: Mechanisms and regulations. *Genes & Development*, 20(7), 759–771. <https://doi.org/10.1101/gad.1410506>.
- Vaucheret, H., (2008). Plant argonautes. *Trends in Plant Science*, 13(7), 350–358. <https://doi.org/10.1016/j.tplants.2008.04.007>.
- Vazquez, F., Vaucheret, H., Rajagopalan, R., Lepers, C., Gascioli, V., Mallory, A. C., Hilbert, J. L., et al., (2004). Endogenous trans-acting siRNAs regulate the accumulation of *Arabidopsis* mRNAs. *Molecular Cell*, 16(1), 69–79. <https://doi.org/10.1016/j.molcel.2004.09.028>.
- Vert, J. P., Foveau, N., Lajaunie, C., & Vandenbrouck, Y., (2006). An accurate and interpretable model for siRNA efficacy prediction. *BMC Bioinformatics*, 7(1), 1–17. <https://doi.org/10.1186/1471-2105-7-520>.
- Voinnet, O., & Baulcombe, D. C., (1997). Systemic signaling in gene silencing. *Nature*, 389(6651), 553. <https://doi.org/10.1038/39215>.
- Voinnet, O., (2009). Origin, biogenesis, and activity of plant microRNAs. *Cell*, 136(4), 669–687. <https://doi.org/10.1016/j.cell.2009.01.046>.
- Voinnet, O., Vain, P., Angell, S., & Baulcombe, D. C., (1998). Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. *Cell*, 95(2), 177–187. [https://doi.org/10.1016/S0092-8674\(00\)81749-3](https://doi.org/10.1016/S0092-8674(00)81749-3).

- Wang, D., Zhang, J., Zuo, T., Zhao, M., Lisch, D., & Peterson, T., (2020). Small RNA-mediated *de novo* silencing of Ac/ds transposons is initiated by alternative transposition in maize. *Genetics*, 215(2), 393–406. <https://doi.org/10.1534/genetics.120.303264>.
- Wang, X., Vignjevic, M., Jiang, D., Jacobsen, S., & Wollenweber, B., (2014). Improved tolerance to drought stress after anthesis due to priming before anthesis in wheat (*Triticum aestivum* L.) var. vinjett. *Journal of Experimental Botany*, 65(22), 6441–6456. <https://doi.org/10.1093/jxb/eru362>.
- Wang, X., Zhang, J., Li, F., Gu, J., He, T., Zhang, X., & Li, Y., (2005). MicroRNA identification based on sequence and structure alignment. *Bioinformatics*, 21(18), 3610–3614. <https://doi.org/10.1093/bioinformatics/bti562>.
- Wani, S. H., & Gosal, S. S., (2010). Genetic engineering for osmotic stress tolerance in plants-role of proline. *IUP Journal of Genetics & Evolution*, 3(4), 14–25.
- Wani, S. H., Singh, N., Devi, T. R., Haribhushan, A., Jeberson, S., & Malik, C., (2013). Engineering abiotic stress tolerance in plants: Extricating regulatory gene complex. In: Malik, C. P., Sanghera, G. S., & Wani, S. H., (eds.), *MD Conventional and Non-Conventional Interventions in Crop Improvement* (pp. 1–21). Publications PVT LTD: New Delhi, India.
- Wassenegger, M., Heimes, S., Riedel, L., & Sanger, H. L., (1994). RNA-directed *de novo* methylation of genomic sequences in plants. *Cell*, 76(3), 567–576.
- Willmann, M. R., & Poethig, R. S., (2007). Conservation and evolution of miRNA regulatory programs in plant development. *Current Opinion in Plant Biology*, 10(5), 503–511. <https://doi.org/10.1016/j.pbi.2007.07.004>.
- Woodrow, P., Pontecorvo, G., Ciarmiello, L. F., Annunziata, M. G., Fuggi, A., & Carillo, P., (2012). Transcription factors and genes in abiotic stress. In: Venkateswarlu, B., Shanker, A., Shanker, C., & Maheswari, M., (eds.), *Crop Stress and Its Management: Perspectives and Strategies* (pp. 317–357). Springer: Dordrecht. [https://doi.org/10.1007/978-94-007-2220-0\\_9](https://doi.org/10.1007/978-94-007-2220-0_9).
- Wu, H., Li, B., Iwakawa, H. O., Pan, Y., Tang, X., Ling-Hu, Q., Liu, Y., et al., (2020). Plant 22-nt siRNAs mediate translational repression and stress adaptation. *Nature*, 581(7806), 89–93. <https://doi.org/10.1038/s41586-020-2231-y>.
- Wu, Y., Wei, B., Liu, H., Li, T., & Rayner, S., (2011). MiRPara: A SVM-based software tool for prediction of most probable microRNA coding regions in genome scale sequences. *BMC Bioinformatics*, 12(1), 1–14. <https://doi.org/10.1186/1471-2105-12-107>.
- Xia, K., Wang, R., Ou, X., Fang, Z., Tian, C., Duan, J., Wang, Y., & Zhang, M., (2012). OsTIR1 and OsAFB2 downregulation via OsmiR393 overexpression leads to more tillers, early flowering and less tolerance to salt and drought in rice. *PLoS One*, 7(1), e30039. <https://doi.org/10.1371/journal.pone.0030039>.
- Xie, F., & Zhang, B., (2010). Target-align: A tool for plant microRNA target identification. *Bioinformatics*, 26(23), 3002, 3003. <https://doi.org/10.1093/bioinformatics/btq568>.
- Xie, K., Minkenberg, B., & Yang, Y., (2015). Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. *Proceedings of the National Academy of Sciences*, 112(11), 3570–3575. <https://doi.org/10.1073/pnas.1420294112>.
- Xin, M., Wang, Y., Yao, Y., Xie, C., Peng, H., Ni, Z., & Sun, Q., (2010). Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (*Triticum aestivum* L.). *BMC Plant Biology*, 10(1), 1–11. <https://doi.org/10.1186/1471-2229-10-123>.
- Xu, J., Chen, Q., Liu, P., Jia, W., Chen, Z., & Xu, Z., (2019). Integration of mRNA and miRNA analysis reveals the molecular mechanism underlying salt and alkali stress

- tolerance in tobacco. *International Journal of Molecular Sciences*, 20(10), 2391. <https://doi.org/10.3390/ijms20102391>.
- Yadav, N. S., Shukla, P. S., Jha, A., Agarwal, P. K., & Jha, B., (2012). The SbSOS1 gene from the extreme halophyte *Salicornia brachiata* enhances Na<sup>+</sup> loading in xylem and confers salt tolerance in transgenic tobacco. *BMC Plant Biology*, 12(1), 1–18. <https://doi.org/10.1186/1471-2229-12-188>.
- Yaish, M. W., Sunkar, R., Zheng, Y., Ji, B., Al-Yahyai, R., & Farooq, S. A., (2015). A genome-wide identification of the miRNAome in response to salinity stress in date palm (*Phoenix dactylifera* L.). *Frontiers in Plant Science*, 6, 946. <https://doi.org/10.3389/fpls.2015.00946>.
- Yao, Y., Ni, Z., Peng, H., Sun, F., Xin, M., Sunkar, R., Zhu, J. K., & Sun, Q., (2010). Non-coding small RNAs responsive to abiotic stress in wheat (*Triticum aestivum* L.). *Functional & Integrative Genomics*, 10(2), 187–190. <https://doi.org/10.1007/s10142-010-0163-6>.
- Younis, A., Siddique, M. I., Kim, C. K., & Lim, K. B., (2014). RNA interference (RNAi) induced gene silencing: A promising approach of hi-tech plant breeding. *International Journal of Biological Sciences*, 10(10), 1150. <http://dx.doi.org/10.7150/ijbs.10452>.
- Younis, M. E., Hasaneen, M. N., & Kazamel, A. M., (2010). Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Vicia faba* seedlings. *Protoplasma*, 239(1–4), 39–48. <https://doi.org/10.1007/s00709-009-0080-5>.
- Yuan, B., Latek, R., Hossbach, M., Tuschl, T., & Lewitter, F., (2004). siRNA selection server: An automated siRNA oligonucleotide prediction server. *Nucleic Acids Research*, 32(suppl\_2), W130–W134.
- Yuan, S., Li, Z., Li, D., Yuan, N., Hu, Q., & Luo, H., (2015). Constitutive expression of rice microRNA528 alters plant development and enhances tolerance to salinity stress and nitrogen starvation in creeping bentgrass. *Plant Physiology*, 169(1), 576–593. <https://doi.org/10.1104/pp.15.00899>.
- Yuan, S., Zhao, J., Li, Z., Hu, Q., Yuan, N., Zhou, M., Xia, X., et al., (2019). MicroRNA396-mediated alteration in plant development and salinity stress response in creeping bentgrass. *Horticulture Research*, 6(1), 1–13. <https://doi.org/10.1038/s41438-019-0130-x>.
- Zeng, J., Ye, Z., He, X., & Zhang, G., (2019). Identification of microRNAs and their targets responding to low-potassium stress in two barley genotypes differing in low-K tolerance. *Journal of Plant Physiology*, 234, 44–53. <https://doi.org/10.1111/nph.13365>.
- Zhang, B. H., Pan, X. P., Wang, Q. L., George, P. C., & Anderson, T. A., (2005). Identification and characterization of new plant microRNAs using EST analysis. *Cell Research*, 15(5), 336–360. <https://doi.org/10.1038/sj.cr.7290302>.
- Zhang, B., (2015). MicroRNA: A new target for improving plant tolerance to abiotic stress. *Journal of Experimental Botany*, 66(7), 1749–1761. <https://doi.org/10.1093/jxb/erv013>.
- Zhang, B., Pan, X., Cannon, C. H., Cobb, G. P., & Anderson, T. A., (2006). Conservation and divergence of plant microRNA genes. *The Plant Journal*, 46(2), 243–259. <https://doi.org/10.1111/j.1365-3113X.2006.02697.x>.
- Zhang, B., Pan, X., Cobb, G. P., & Anderson, T. A., (2006). Plant microRNA: A small regulatory molecule with big impact. *Developmental Biology*, 289(1), 3–16. <https://doi.org/10.1016/j.ydbio.2005.10.036>.
- Zhang, H., Zhang, H., Demirer, G. S., Gonzalez-Grandio, E., Fan, C., & Landry, M. P., (2020). Engineering DNA nanostructures for siRNA delivery in plants. *Nature Protocols*, 15(9), 3064–3087. <https://doi.org/10.1038/s41596-020-0370-0>.
- Zhang, J. L., & Shi, H., (2013). Physiological and molecular mechanisms of plant salt tolerance. *Photosynthesis Research*, 115(1), 1–22. <https://doi.org/10.1007/s11120-013-9813-6>.

- Zhang, S., & Hong, Z., (2019). Mobile RNAs—the magical elf traveling between plant and the associated organisms. *ExRNA*, 1(1), 1–6. <https://doi.org/10.1186/s41544-019-0007-z>.
- Zhang, W., & Xie, B., (2017). A meta-analysis of the relations between blood microRNA-208b detection and acute myocardial infarction. *Eur. Rev. Med. Pharmacol. Sci.*, 21(4), 848–854.
- Zhang, X., Zou, Z., Gong, P., Zhang, J., Ziaf, K., Li, H., Xiao, F., & Ye, Z., (2011). Over-expression of microRNA169 confers enhanced drought tolerance to tomato. *Biotechnology Letters*, 33(2), 403–409. <https://doi.org/10.1007/s10529-010-0436-0>.
- Zhang, Y., (2005). miRU: An automated plant miRNA target prediction server. *Nucleic Acids Research*, 33(suppl\_2), W701–W704. <https://doi.org/10.1093/nar/gki383>.
- Zhang, Z., Teotia, S., Tang, J., & Tang, G., (2019). Perspectives on microRNAs and phased small interfering RNAs in maize (*Zea mays* L.): Functions and big impact on agronomic traits enhancement. *Plants*, 8(6), 170. <https://doi.org/10.3390/plants8060170>.
- Zhang, Z., Yu, J., Li, D., Zhang, Z., Liu, F., Zhou, X., Wang, T., et al., (2010). PMRD: Plant microRNA database. *Nucleic Acids Research*, 38(suppl\_1), D806–D813. <https://doi.org/10.1093/nar/gkp818>.
- Zhao, C., Zhang, H., Song, C., Zhu, J. K., & Shabala, S., (2020). Mechanisms of plant responses and adaptation to soil salinity. *The Innovation*, 1(1), 100017.
- Zhao, F. J., Ma, J. F., Meharg, A., & McGrath, S., (2009). Arsenic uptake and metabolism in plants. *New Phytologist*, 181(4), 777–794. <https://doi.org/10.1111/j.1469-8137.2008.02716.x>.
- Zhao, X. Y., Hong, P., Wu, J. Y., Chen, X. B., Ye, X. G., Pan, Y. Y., Wang, J., & Zhang, X. S., (2016). The tae-miR408-mediated control of TaTOC1 genes transcription is required for the regulation of heading time in wheat. *Plant Physiology*, 170(3), 1578–1594. <https://doi.org/10.1104/pp.15.01216>.
- Zhou, J., Liu, M., Jiang, J., Qiao, G., Lin, S., Li, H., Xie, L., & Zhuo, R., (2012). Expression profile of miRNAs in *Populus cathayana* L and *Salix matsudana* Koidz under salt stress. *Molecular Biology Reports*, 39(9), 8645–8654. <https://doi.org/10.1007/s11033-012-1719-4>.
- Zhou, M., & Luo, H., (2013). MicroRNA-mediated gene regulation: Potential applications for plant genetic engineering. *Plant Molecular Biology*, 83(1, 2), 59–75. <https://doi.org/10.1007/s11103-013-0089-1>.
- Zhou, M., & Luo, H., (2014). Role of microRNA319 in creeping bentgrass salinity and drought stress response. *Plant Signaling & Behavior*, 9(4), 1375–1391. <https://doi.org/10.4161/psb.28700>.
- Zhou, M., Li, D., Li, Z., Hu, Q., Yang, C., Zhu, L., & Luo, H., (2013). Constitutive expression of a miR319 gene alters plant development and enhances salt and drought tolerance in transgenic creeping bentgrass. *Plant Physiology*, 161(3), 1375–1391. <https://doi.org/10.1104/pp.112.208702>.
- Zhu, X., & Galili, G., (2004). Lysine metabolism is concurrently regulated by synthesis and catabolism in both reproductive and vegetative tissues. *Plant Physiology*, 135(1), 129–136. <https://doi.org/10.1104/pp.103.037168>.
- Zhuang, Y., Zhou, X. H., & Liu, J., (2014). Conserved miRNAs and their response to salt stress in wild eggplant *Solanum linnaeanum* roots. *International Journal of Molecular Sciences*, 15(1), 839–849. <https://doi.org/10.3390/ijms15010839>.
- Zuo, L., Wang, Z., Tan, Y., Chen, X., & Luo, X., (2016). piRNAs and their functions in the brain. *International Journal of Human Genetics*, 16(1, 2), 53–60. <https://doi.org/10.1080/09723757.2016.11886278>.

**PART III**  
**Epigenetics and Omics Approaches**





# Taylor & Francis

Taylor & Francis Group

<http://taylorandfrancis.com>

## CHAPTER 10

---

# Genome-Wide Association Studies and Next-Generation Sequencing in Plant Response to Environmental Stress

POOJA SARASWAT, HUNNY WASWANI, and RAJIV RANJAN\*

*Dayalbagh Educational Institute, Department of Botany, DayalBagh,  
Agra – 282005, Uttar Pradesh, India*

*\*Corresponding author. E-mail: rajivranjanbt@gmail.com*

---

### ABSTRACT

The increasing global demand for food and the betterment of agriculture is a necessary need that is to be resolved. Due to the extreme climate changes and environmental stress, plant growth and productivity suffers a lot. To find solution to these problems, there is an obvious need to develop methods and techniques to identify the genetic variations among any crop plant's genomic structure that are resilient to environmental changes. So, that breeding of new crop varieties becomes easier. The newly developed techniques like genome-wide association study (GWAS) are prominently accepted as to recognize genotypes to their respective phenotypes. GWAS is based on the different statistical methods to determine the genetic variations associated with phenotypes in any population. On the other hand, Next generation sequencing (NGS) is also helping side by side in providing all the required information of novel genes and their expression in response to environmental stresses. The identified QTLs and SNPs associated with the genes help in future enhancement of plant biology and agriculture. The combined efforts made by researchers and the new techniques are helping in boosting

of breeding strategies. This chapter covers the importance of GWAS and NGS in crop improvement against various biotic and abiotic stresses.

## **10.1 INTRODUCTION**

The pace of conversion of arable land to wasteland is increasing due to the rising global population, urbanization, and industrialization. One of the main issues that agriculturalists and the plant scientists are currently facing is the supplying food to an ever-increasing population. This predicament is made considerably worse by environmental pressures. Despite the development of a number of tolerance mechanisms, plants that are sensitive to environmental extremes have a hard time surviving. It is critical to develop new technological approaches. Plant genomes have little ability to improve resistance to environmental stress using traditional breeding strategies. Plants are in direct contact with a vast range of environmental stresses as it lowers and control the growth and productivity of many plants. The plants are consistently in direct contact with different kinds and various forms of stress. The identification of critical genes and signaling pathways underpinning plant responses to environmental stress will aid in the development of strategies for agricultural genetic improvement to address this problem. Abiotic stress-related genes have been identified in large part thanks to crop functional genomics. Recent advancements in genomic technologies now enable cost-effective solutions. Especially with the availability of the entire genomic sequence of several model and agricultural plant species, and high-throughput technologies for discovering stress-related genes at a genome-wide level. Bioinformatic approaches have been able to uncover stress-tolerant gene families across species based on homology and synteny. Thanks to the availability of genetic database resources. Furthermore, genome-wide association analyzes (GWAS) for complex trait loci in crops have aided in the finding of important stress-related genes and their beneficial alleles.

Due to the development of next-generation sequencing (NGS) technologies, GWAS has become a generally acknowledged technique for deciphering genotype-phenotype relationships in many species. The overall purpose of GWAS is to use the most appropriate statistical model in a given population to link genotypic variants to phenotypic differences. Genome-Wide Association studies (GWAS), or we can define as the studies which are associated with the genome. They investigate genetic variants across un-identical varieties, at the genomic level. To know that these variants are associated

with traits or not, GWAS mainly focuses on connections between traits and SNPs (Manolio, 2010). GWAS is considered as a powerful and ubiquitous tool which has been used in the study of complex traits. GWAS is being effectively and efficiently used for genome–phenotype association studies and used to investigate or in identifying a disease either in plant or animal.

Although NGS technology is still in its early stages of development, it has proven to be a reliable method for identifying genetic diversity across the genome. Single nucleotide polymorphisms (SNPs), insertions, and deletions, and copy number changes, all of which are linked to growth and development as well as stress responses. SNPs, insertions, and deletions, and copy number changes are all examples of genetic variants. NGS has been used in conjunction with GWAS to find potential molecular markers. The research of comparative genomics is aided by NGS technology, which allows studies of variety within and across species discussed later in the chapter.

## 10.2 PLANT RESPONSE TO VARIOUS ENVIRONMENTAL STRESSES

Stresses are generally occurred by the conditions in which the plants are placed, and these stresses affect the plant's growth and mechanisms (Verma et al., 2013) that lowers the plants productivity. The major alterations are done in the healthy plant by different stresses and these alterations trigger the most of the responses like changes in the growth and development rate of the plant, their gene expression, altered cellular metabolism, and yields of plants or crops. Plants usually react to the changed environment and these sudden changes in plants show the restored environmental conditions or we can describe that plants are encountered with this diverse range of environmental stresses, which ultimately affect their productivity. The plant stresses are majorly categorized into two types: (i) abiotic stresses; and (ii) biotic stresses. The abiotic stresses cover two major subunits physical and chemical. Light, water, salinity temperature comes under physical subunits. While, the pesticides, pollutants, and toxic heavy metals (Seneviratne et al., 2019) are some of the chemical forms of stress. Whereas the insecticides, diseases (Verma et al., 2013), capturing through herbivorous animals (Morkunas et al., 2018) majorly comes in the biotic form of stresses. Both the stresses show different impacts on plants, but salinity, temperature, and drought impose major impacts (Figure 10.1). These adversely affect not only their growth and productivity, but they destroy or injured them to their depth, because of which their metabolic dysfunctioning starts (Verma et al.,

2013). Plants are so adaptable to this environment so they cannot escape, as it has become their compulsion to remain in that environment. Their senses become active at the time of stress and then they generate the cellular responses according to that stress but to fulfill their lifecycle they have to retain themselves within the stress conditions. For survival in these adverse conditions, numerous mechanisms are developed by plants to overcome the stress condition. Some plants themselves recover through stresses if the duration of the stress is short or the effect of that stress is low or mild, but in severe conditions the death of plants occur and this creates severe impact on the crop productivity (Verma et al., 2013). Although this entire works upon the types of plant as we have seen the desert plants, they can bear such kind of stress altogether (Zhu, 2002).

### **10.2.1 ABIOTIC STRESS**

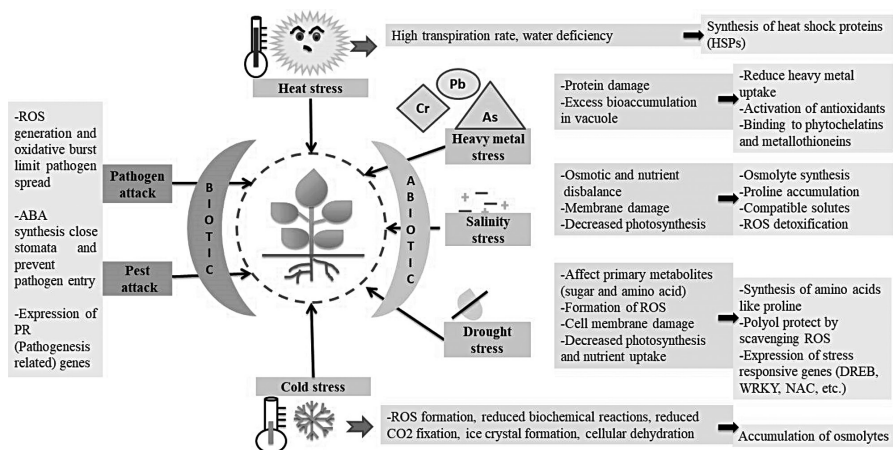
- 1. Drought (Water Stress):** The climate change all around the world making a rise in temperature, as well as in the levels of hazardous gases, such as CO<sub>2</sub>, which is ultimately creating a disturbance in the environment and the geological cycles. Uneven rains which result in the cause of drought, plants growth becomes steady, and this is the first response of plants in the drought areas, where the amino acids and carbohydrates starts accumulating and plays their role in the osmoprotection. Main amino acids like valine, leucine, isoleucine, and agmatine works as a precursor in polyamines which provide the preservation of cellular integrity, control the inhibition or growth through stress we can say that this is the primary stress as it affects the plant's physiology. Short term drought in which carbohydrates alcohols are synthesized and this is not in the control of ABA signaling but when there are long term drought stress amino acids are produced which are in control of ABA signaling (Breckle, 2002).
- 2. Salinity (Salt Stress):** The accumulation of salt is a matter of concern nowadays, as the large area of land is becoming saline globally usually every year, and it is expanding day by day, in agricultural land it is becoming a threat, because it is reducing the crop yield. Salt gets accumulated near the root zone of the plants which is declining the productivity. Salinity stress does not mean that the concentration of salt is high in the soil, it means that the soil is also containing two major primary effects also which is osmotic stress and the ion

toxicity. The osmotic stress hikes whenever there is a saline soil because the soil itself is high in the salts, so it reduces the uptake activity of minerals and water like calcium and potassium. In salt stress, the plant which cannot tolerate the stress starts showing arrest in their growth and in their reproduction with organ detachments. As well as some acids and compounds get accumulated in plant such as the jasmonic acid, polyphenolic compounds (Pedranzani et al., 2003). Levels of anthocyanin also decrease wherever there is a salt stress, or the land is saline (Daneshmand et al., 2010).

3. **Extreme Temperature (Cold, Heat):** This stress always shows up a negative impact whether the temperature is high or low, i.e., heat stress or cold stress. It directly creates an impact on the plant metabolism. Many drastic effects are observed under temperature change, it affects the stabilization of proteins or premature leaf senescence, efficiency of enzymes, and cytoskeleton structure (Morison & Lawlor, 1999). The rate of seed germination photosynthetic efficiency decreases when the heat stress come across, physical alterations are done in the plasma membrane of a cell, which damages the electron transport chain, ATP, oxygen-evolving complex and carbon assimilation mechanism (Scholz et al., 2004). Talking about the low temperature or cold stress it also creates multiple effects on the plants. A limiting factor is created by the cold stress ranging from temperature (0–15°C) which is beyond freezing, it ceases the growth of plant, done by reducing the amplitude in energy utilization which ultimately does a reduction in the rate of photosynthesis and production of reactive oxygen species.

### **10.2.2 BIOTIC STRESS AND PLANTS RESPONSES**

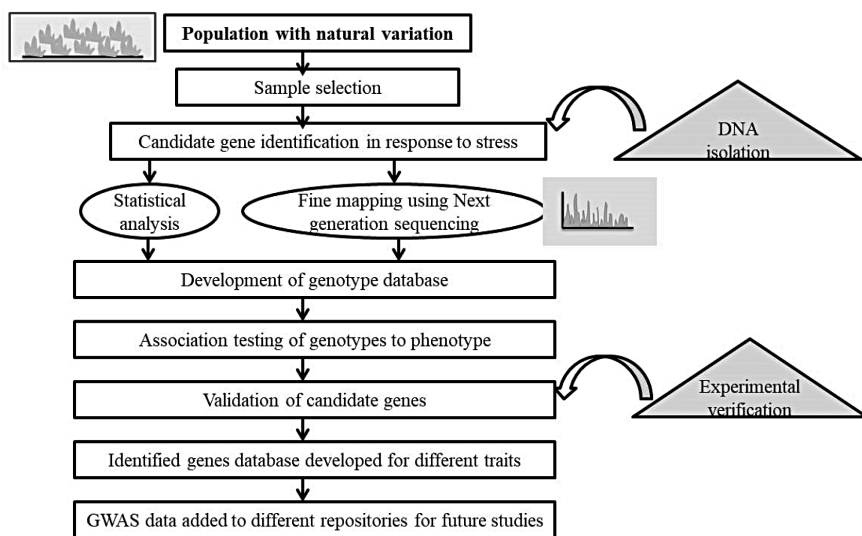
The biological factors are altering these stresses, such as diseases and insects, which alters the growth the growth of plant throughout their life span. Plants usually react to these stresses by developing secondary metabolites, sudden changes in their cell walls, etc. To stop the functioning of harmful pathogens or to isolate them on the other hand they are stopping their spreading across the plant. These sudden changes are known as the Hypersensitive Reactions. Polyamines accumulation shows that there is a sudden change which is occurring in the plant or there is an interaction of pathogens whether it is viral, fungal (Torrighiani et al., 1997; Asthir et al., 2004).



**FIGURE 10.1** The effect of environmental stress on plants and their response to the stressed conditions.

### 10.3 GENOME WIDE ASSOCIATION STUDIES IN PLANTS

The overgrowing computational methods and sequencing technologies have made GWAS a powerful tool for detecting natural variations that comes under complex traits in crops (Rafalski, 2010). Based on linkage disequilibrium, GWAS is a method for high-resolution mapping of complex trait loci (Flint-Garcia et al., 2005). The connection between genetic variation and crucial agricultural properties, such as stress tolerance, has been studied using GWAS. Its potential for uncovering beneficial natural variants in trait-associated loci is still in its infancy. As well as allelic variants in candidate genes that underpin quantitative and complex variables, such as growth, development, stress, and nutritional quality. The priority assigned to genes selected for more intensive biochemical and physiological investigations on the processes of stress response can be defined using candidate genes identified by GWAS. In crops, GWAS use an everlasting resource that is the population of different varieties can be genotyped once but can be rephenotyped many times and can generate mapping populations for specific traits or QTLs in crops (Atwell et al., 2010). A general workflow of GWAS is demonstrated in Figure 10.2. On the other hand, GWAS adopts a case-control design in humans, i.e., the comparison between wide groups of patients and healthy people can be identified with the help of population-scale genome design that suspects loci of a particular disease. The missing heritability problem has inclined the GWAS of humans as they identify the loci that have a very low rate of phenotypic contribution.



**FIGURE 10.2** The workflow of the process used in genome-wide association studies.

GWAS in crops are much less costly than that of humans because high population is required to detect the QTLs among humans than that of crops and the number of markers should increase continually. Crops like maize, rice, canola, wheat, sorghum, etc., has approved GWAS (Chen et al., 2017; Tessmann et al., 2018; Raman et al., 2019; Neang et al., 2020). In the reference of rice, 1,083 cultivated varieties and 446 wild varieties were sequenced and observed with the low genome coverage (Huang et al., 2012). In maize, linkage mapping and GWAS were used together in the NAM panel in which the genetic structural design of flowering time, leaf angle, leaf size and disease resistance traits have been dissected (Buckler et al., 2009; Kump et al., 2011; Poland et al., 2011; Tian et al., 2011). In crops GWAS include weighing the trade-offs of increased false-negative and decreased false-positive rates that helps in defining the structure of a particular crop (Korte & Farlow, 2013; Myles et al., 2009; Platt et al., 2010; Vilhjalmsen et al., 2012).

In detecting the genotype and phenotype association in crops the most favorable method is the mixed model (Bradbury et al., 2007; Yu et al., 2006). Advancements in these models has significantly lowered the computational time or calculation time and this is possible by EMMAX program, i.e., Efficient Mixed-Model Association expedited and the compressed mixed linear model method (Kang et al., 2010; Lipka et al., 2012; Lippert et al., 2011; Zhang et al., 2009, 2010; Zhou et al., 2012).



### **10.3.1 PRINCIPLE OF GWAS**

To lead a GWAS test, the initial step is to choose the population of study with a full thought of the size of the population (least 100 people) with inclination to expand the number of people however much as could be expected to stay away from Beavis impacts that lead significantly overestimated of phenotypic change when the number of people are little for example 100 (Xu, 2003). Then, at that point, there are three significant stages for playing out a fruitful GWAS explore:

- **Stage I:** It is the phenotyping where all genotypes ought to be phenotype for a specific characteristic or gathering of attributes dependent on the targets of the investigation. Exact phenotyping is a basic highlight identifies genotype-phenotype affiliations. Phenotyping ought to be rehashed over replications as well as areas and additionally years. The wide sense heritability ought to be determined for crude information (note, it ought to be determined subsequent to eliminating the outliers) including these components and thinking about  $G \times E$  cooperation. High heritability is a marker that the characteristic is for the most part hereditarily controlled which is critical to identify the affiliation signals. Then, at that point, the phenotypic information can be utilized to appraise the mean for example BLUE or BLUP. Since the phenotypic information are exceptionally lopsided in the plants, the assessment of genotypic qualities is for the most part determined as fixed impacts (for example BLUE) utilizing blended models (Piepho et al., 2007), which have been effectively utilized in grain (Milner et al., 2019; Thabet et al., 2018; Nagel, 2018; Saade et al., 2016).
- **Stage II:** It is the genotyping wherein a similar arrangement of individual that were phenotyped is utilized for genotyping utilizing DNA molecular markers. GBS is the most continuous technique utilized in genotyping on the grounds that it creates various SNP markers economically that covers the yield genome (for example wheat, grain, and so on). The GBS-created SNPs ought to be shifted dependent on missing information, heterozygosity, and minor allele frequency. Prior to running GWAS, construction of population GWAS model should be done in an understandable form.

The statistical models like the general linear model (GLM) and mixed linear model (MLM) regularly proposed for performing

GWAS. The GLM doesn't take the populace structure are not taken in GLA model. Henceforth, GLM was utilized in populaces which didn't have the populace structure in faba bean, *Vicia faba* L. (Sallam & Maure, 2016) and rice (Bandillo et al., 2013). The MLM, then again, thinks about the populace structure in its model. At last, the phenotypic and genotypic information are joined utilizing proper programming (TASSEL) by which alleles related with a specific characteristic can be recognized after the GWAS model was chosen.

- **Stage III:** Phenotyping is strongly recommendable to be led prior to genotyping, particularly for those populaces with no earlier data. For instance, if a populace comprised of 400 genotypes which were gathered from various locales and the objective is to test them in a specific climate. It is conceivable that numerous genotypes could be lost because of helpless variation to the phenotyping climate. Along these lines, time, and currency (for genotyping) can be saved by testing the phenotypic variety of that populace first.

#### 10.4 NEXT GENERATION SEQUENCING (NGS)

Initial attempts to obtain genetic sequences necessitated significant human and financial resources. However, the development and implementation of next-generation sequencing (NGS) technology has substantially aided the capacity to produce genome sequences for an increasing number of plant species. This has opened up a slew of possibilities for finding stress-related genes and pathways that can serve as the foundation for future research or for the purpose of crop improvement. NGS technology, for example, can be combined with high-throughput transcriptome profiling to explore transcriptome-wide changes in response to stress (Molina et al., 2011). Inexpensive technology of sequencing, often it is known as next generation sequencing (NGS) technologies, its main feature is that at once it constructs millions of sequencing reads (Church, 2006). NGS is a rapid technology which works in a very cost-effective way for constructing large quantity of sequence data. In addition, it is useful in many fields that are in comparative genomics where we compare between genomes of species, data through NGS have higher capability in identifying the loci under selection. The new terminologies which define NGS are (high throughput sequencing, massively parallel sequencing, and deep sequencing). It's the advanced and modern technology which came in existence last decade. NGS technologies bring a huge change

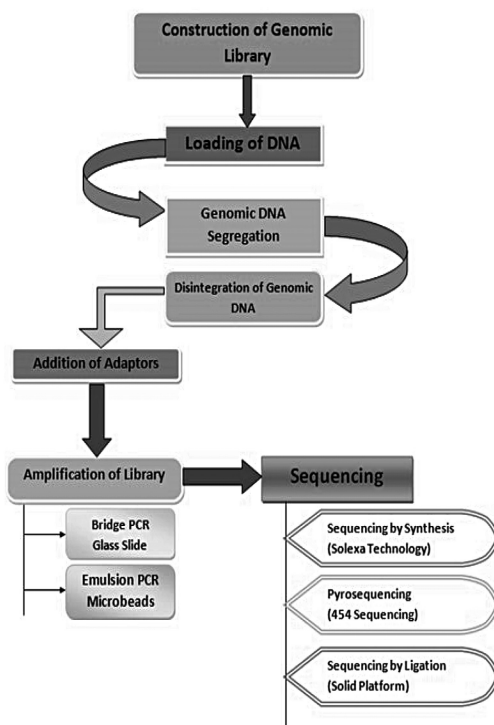
in or transformed this living system or the world of biology and that's not enough, it reorganized the biomedical world too. Representation of NGS was very first showed up in 1996 (Ronaghi et al., 1996). This methodology is highly developed, and currently, it is in use, and it is governing by various platforms like Illumina and Life Technologies. The advanced sequencing technologies reduces the expenditure of sequencing, on the other hand the most up to date sequencing technologies not only lowers the sequencing cost, also they are very quick which trim down the time required for sequencing (Schuster, 2008; Tucker et al., 2009).

#### **10.4.1 PRINCIPLE OF NGS**

Principle of NGS is quite relatable to the previous sequencing technologies or traditional sequencing, Sanger sequencing method that consider capillary sequencing, is known as first generation sequencing. Generally, various NGS stages embrace their own unique methodology of sequencing. Beginning of NGS is done by preparing a template, the first step. And it all starts with a Double-Stranded DNA material. Or we can say that DNA is the raw material. Sources or isolation of this DNA material can differ. We can take different DNA as sources they can be immuno-precipitated DNA, Reverse transcribed DNA, or cDNA (Rizzo et al., 2012). The second step of this technique is the preparation of library correspondingly. As we said that we use different DNA so as their work also differs, and we use them accordingly. Fragmentation is to be done into linear DNA molecules if we are considering genomic DNA as a first step. cDNA is acquired in the making of linear DNA molecules, and this is only possible or only done in the case of RNA where RNA is used in the preparation by the help of reverse transcription (Figure 10.3). The major differences between the first-generation sequencing and second-generation sequencing are DNA fragments are used in lesser quantity, only one DNA fragment is sufficient.

#### **10.5 GWAS IN UNDERSTANDING PLANT RESPONSE TO ENVIRONMENTAL STRESS**

The common methods used for the detection of multiple traits in plants are QTL mapping and GWAS. Quantitative trait loci (QTL) mapping is generally a statistical way to link two different sets of data including phenotypes and genotypes. On the other hand, GWAS or linkage disequilibrium (LD)



**FIGURE 10.3** Workflow of the process of next generation sequencing (NGS).

mapping takes advantage of linkage disequilibrium to find a link between phenotypes and genotypes. The differentiation of genotypes is done on the basis of molecular markers and phenotypes on the basis of traits in a reference set of germplasm. Theoretically, GWAS can be used in any germplasm set for the identification of QTL that reveal variation in different traits (Malosetti et al., 2007). A QTL is basically a part or region of DNA that is related to some trait phenotype. The reason why GWAS is used is due to its high-resolution mapping which provides greater capability in the identification of rare alleles by using statistical measures (Jin-long et al., 2012). Different molecular markers like amplified fragment length polymorphism (AFLPs) and SNPs are in use for mapping of QTLs which are further correlated with the phenotypic data. With QTL mapping, there is an advantage that different variants can be mapped in F<sub>2</sub> generation of recombinant inbred line (RIL). But this advantage also ends up with its limitation as the mapping

can be done up to RIL population (Korte & Farlow, 2013). To overcome the drawbacks of QTL mapping, GWAS was introduced. GWAS is basically free from any hypothesis but can generate hypothesis. Also, GWAS can be used in the identification of more variants related to any trait. In any GWAS study, the SNPs present in the sequence data are used to correlate different genetic variants, phenotypes, etc., in any population of organism. Other similar approaches investigate a particular portion of the chromosome while GWAS is able to explore the whole genome. GWAS has been used in various biotic and abiotic stress responses in the plants discussed later in the chapter. Different stresses are controlled by various genes like biotic is mostly controlled by a single gene while abiotic stress is based on multiple genes (Wan et al., 2017). So far, many studies have been done using GWAS mentioned in Table 10.1.

### **10.5.1 ABIOTIC STRESS**

Abiotic stress affects the plant development and growth by affecting plant metabolism and cellular pathways. To understand the effect of genes responsible for the stress response, such putative genes need to be identified from population of any organism. For this several studies based on GWAS have been performed recently in response to abiotic stresses. A study has been reported in which the identification of genes responsible for drought resistance in rice plant was observed with an aim to develop tools that can be used in future rice breeding. For the analysis of 175 rice accessions have been done with variable water availability. As a result, 13 SNP markers related to the yield under drought were identified and the stepwise regression analysis, 8 SNP markers were validated by *in silico* methods. The PCR validation revealed that two SNP markers have been found similar to the genotypes with higher yield under water deficit conditions. This drought experiment also showed that 30 genes out of 50 were annotated and 10 (e.g., WRKY TFs and other enzymes) have been reported as linked to drought and other abiotic stresses (Pantaliao et al., 2016). Arbuscular mycorrhizal symbiosis has a typical role in the improvement of drought stress resistance in wheat. But the related QTLs associated in response to drought stress are not well known. Therefore, a GWAS was conducted to reveal such QTL regions responsible for controlling the stress response against low water conditions. Such studies will help in future work related to the gene identification too (Lehnert et al., 2018).

**TABLE 10.1** List of Some Plants for Which GWAS has been Used to Identify Genes in Response to Stress

Plant	Study Done	Trait Identified/Possible Outcomes	References
<i>Arabidopsis thaliana</i>	Low water potential induced proline accumulation	Genes related to cellular metabolic and redox status	Verslues et al. (2014)
<i>Oryza sativa</i>	To study salinity tolerance	New QTLs related to stress tolerance	Kumar et al. (2015)
<i>Oryza sativa</i>	Grain yield under drought condition	Genes related to drought tolerance were identified	Pantaliao et al. (2016)
<i>Sorghum bicolor</i>	Heat tolerance during vegetative stage of growth	Gene directly or indirectly linked to the pathways involved in heat tolerance were identified	Chen et al. (2017)
<i>Medicago sativa</i> L.	To map loci associated with plant growth under salt stress	Putative candidate gene linked to marker loci related to salt tolerance	Liu et al. (2017)
<i>Brassica napus</i> L.	Salt tolerance related QTL	38 possible genes identified	Wan et al. (2017)
Soybean	Role in regulation of photosynthesis	Photosynthesis	Wang et al. (2020)
Camelina	To study seed germination under salt stress condition	17 SNPs were identified for metabolism and cellular activities	Luo et al. (2020)
Rice	To find SNPs related to salt removal in leaf sheath of rice	Genes associated with Na <sup>+</sup> removal in leaf under salinity conditions	Neang et al. (2020)
Rice	For early-stage salt tolerance	QTLs for salt stress tolerance	Nayyeripasand et al. (2021)
Rice	To identify loci associated with sulfur deficiency	Loci associated with root and root length was identified. Genes associated with loci for (sulfotransferases) important in S metabolic pathways	Pariasca-Tanaka et al. (2020)
<i>Triticum aestivum</i>	In artificial warm treatment	Fusarium head blight	Tessmann et al. (2018)
<i>Wheat</i>	For multiple biotic stress resistance	Significant marker-trait associations (MTAs) identified for multiple biotic stresses	Bhatta et al. (2019)

Oladzad and coworkers (2019) conducted a single and multiple-trait GWAS in which four genes associated with production traits have been identified common bean in relation to abiotic stress (heat and drought). Nucleotide polymorphism found within or in the nearby candidate genes which were related to the signaling and detoxification of ROS were identified which can further be used in the breeding of common bean. *Brassica juncea* has been studied for terminal heat stress (THS) using GWAS as to improve productivity in the changing climatic conditions. A total of 491 genotypes were accessed for the study to understand the variations under natural heat stress conditions. 18,258 SNPs were identified from 71 genotypes after the filtration process. Later, 24 SNPs under THS were identified which were associated with traits related to seed yield. This was the first reported study in which 24 marker traits were recognized under terminal heat stress condition (Sandhu et al., 2019).

GWAS was employed in the identification of two new loci responsible for photosynthetic traits which is associated with the efficiency of phosphorus (P) in soybean. As P is important for plant's growth and development, it plays a vital role in acquiring energy, enzyme regulation and yield. Therefore, soil with P deficiency inhibits the production and yield of any crop. To evaluate the efficiency of P, 219 soybean accessions from three environments were taken. As a result, 30 notable SNPs found in 14 different regions of the genome were observed which were associated with traits related to photosynthesis. The observation was done under different phosphorous levels (Yang et al., 2020). Similarly, deficiency of sulfur is one of the main causes in reduction of grain quality and yield in rice. Therefore, the genotypic variation against resistance to sulfur deficiency has been assessed using GWAS and loci associated with tolerance has been identified. The genes identified near the loci associated were found in the coding of enzymes (sulfotransferases) related to metabolic pathways, and also the role of sulfated compounds was recognized against abiotic stress responses. Further, the haplotypes obtained could make use in marker assisted breeding to develop cultivars that can tolerate S-deficiency (Pariasca-Tanaka et al., 2020).

A transcriptome based GWAS analysis has been performed to understand the traits involved in seminal root length under drought condition in the maize seedlings. In this study, 209 maize accessions were taken under different water conditions and as a result four traits associated with root and shoot were evaluated. To identify the genes for drought response, the comparison of transcriptome results of seminal roots for n four drought-sensitive and drought-tolerant lines has been done under different water conditions. After

the drought stress was given 343 differentially expressed genes (DEGs) were identified in drought-tolerant group and 177 in the drought-sensitive group. In the end, 7 putative genes were considered for further experiments and also the results add on to knowledge regarding seminal root changes under drought conditions in maize (Guo et al., 2020). Photosynthesis is an important process in the life of the plant as it is directly linked to yield and productivity. Therefore, improvement in photosynthesis is important to increase crop productivity. In a study, SNPs were found associated with different parameters of photosynthesis including stomatal conductance (Cond), net photosynthetic rate (Pn), transpiration rate (Trmmol) and intercellular carbon dioxide concentration (Ci) by using GWAS in soybean. Around 219 soybean accessions were exploited in which 12 QTLs linked to different traits like Ci, Pn, Trmmol and Cond were disclosed (Wang et al., 2020). As GWAS is able to do fine mapping of QTLs in a population, it is also used in plant response to salt stress in the early stage of growth (vegetative). Nayyeripasand and co-workers (2021) used more than 33,000 SNP markers to identify regions in the rice genome associated with salt tolerance in vegetative stage.

There are several online tools available which help in integration of GWAS and candidate gene information, understanding of pathways, interpretation of genomic data, etc. TraitCapture is an online tool which helps in the rapid identification of candidate genes, provides high throughput phenotypes associated with genomes, next generation, and environment monitoring data. It combines both GWAS and FSPMs to identify phenotypes in any particular environment condition (Brown et al., 2014). Some other tools are available for GWAS related data including PostGWAS, SNPinfo, PAST (Pathway association tool), GMStool (GWAS-based marker selection tool, etc. (Xu & Taylor, 2009; Hiersche et al., 2013; Thrash et al., 2020; Jeong & Kim, 2020).

### **10.5.2 BIOTIC STRESS**

The appearance and re-appearance of plant pathogens bring deviation in the process of both pathogen and host. It becomes necessary to understand the genetic variations among the natural variations to know the adaptability of the wild plant and make it feasible to improve breeding of resistant types. The present time of genomics has led to the emergence of statistical tools like GWAS important in understanding and discovery of novel genes associated with desired traits which may include genes for resistance to environmental stress. However, GWAS is not much used for estimation of genetic variants in relation to pathogen resistance.



The first study based on GWAS showing the interaction of bacteria *P. syringae* was performed in 2005 by Aranzana and colleagues (2005). Recently, the enormous use of GWAS is due to the new technique developed, i.e., NGS. This new technique is used for fine mapping of natural variation using genetic markers (Bartoli & Roux, 2017). As tolerance to biotic stress is the ultimate goal of breeders. A study done on hexaploid wheat plant under different biotic stress has been reported in which 125 hexaploid wheat were identified in providing resistance to fungal infections that causes various diseases (wheat rusts and crown rot). The GWAS study confirmed the genes and the genomic regions supporting resistance to biotic stress as 124 marker-trait associations (MTAs) were estimated, out of which 33 of them were found within the gene sequences. The result provided more insights into stress resistance in wheat for future breeding programs (Bhatta et al., 2019).

## **10.6 ROLE OF NGS IN UNDERSTANDING PLANT RESPONSE TO ENVIRONMENTAL STRESS**

The next-generation sequencing or NGS technologies is a new technique which includes Illumina/Solexa's sequencing technology (San Diego, California, USA) (Quail et al., 2008), and SOLiD sequencing technology, etc. NGS has wide applications in the field of genomics like genome projects, transcriptome analysis and RNA sequencing. The advance in NGS technology has enabled researchers to identify gene networks of candidate genes that may response to different environmental stress. The understanding of genetic variations of secondary traits related to stress tolerance and identification of variations in nucleotides helps breeders to improve crops efficiently. In recent years, the role of GWAS in the detection of genes associated with developmental processes like stress tolerance in plants has been explored with more clear vision about the expression patterns under stress conditions in different parts and stages of the plant (Yu et al., 2006). The NGS-based RNA-seq methods for transcriptomes can help in the characterization of genes, detection of gene expression, identification, and quantification of new transcripts and variation in sequence of identified genes. Studies suggest that the response to various stresses is controlled by a wide range of regulatory genes and their related mechanisms. Transcription factors (TFs), other genes coding for transport proteins that are somewhere involved in metabolic pathways and signaling gets activated under stress conditions (biotic and abiotic) (Atkinson & Urwin, 2012; Fan et al., 2013). However, unique genes are activated against

different stress (Atkinson & Urwin, 2012). Like for example, under heavy metal concentrations, the plant withstands the situation by adopting some common ways of defense which may include detoxification of excess heavy metals. This detoxification process may include generation of ion sensing, activation of metal transporter proteins and regulation of TFs as needed. The regulation mechanisms and the genes associated with response to stress have been effectively identified using NGS technologies.

Lead (Pb), being a toxic heavy metal, is accumulated by plants roots which result in the entry of lead in the food chain which causes health issues in human beings. In a study, NGS based-RNA sequencing was employed to estimate the DEGs in radish plant and their effects under Pb stress were examined (Wang et al., 2013). Another similar study has been reported on Chromium (Cr) stress response in radish. The insufficient knowledge about the molecular mechanism in response to Cr stress makes it more important to be understood. In a study GWAS based RNA-Seq has been employed in the identification of DEGs in response to Cr stress. The study resulted in finding new insight to understand molecular mechanism in response to Cr stress which helps in future manipulations in the genes for Cr accumulation in radish plants (Xie et al., 2015). The microRNAs (miRNAs) are responsible for growth, development, and response to environmental stress in plants. The miRNAs are basically non-coding RNA sequences which are single-stranded. The role of miRNA in heat stress response in radish has been reported but their characterization is still unclear. So, as to understand the effect of miRNA on target genes, Wang and colleagues (2015) identified 26 previously known and 19 new miRNAs under heat stress.

*Phytophthora infestans* (Mont.) is the causal pathogen to spread the destructive disease in Tomato. Wild tomato is known to possess genes for the resistance against this pathogen which could be used as a reference to develop better breeds of tomato. To find such genes an identification study has been done by Arafa and coworkers (2017) to find the candidate genes that may provide resistance to tomato late blight by applying NGS technologies. Two genes namely, Solyc06g083640.3 and Solyc06g071810.1 were taken as candidates against resistance to disease. It was concluded that the SNP and SSR markers linked to these two genes can be further used in marker-based selection for breeding plants resistant to late blight disease. Betaine aldehyde dehydrogenase 1 (BADH1), is a gene associated with salt stress which is therefore involved in abiotic stress response. In the early stage of germination, 475 rice accessions were used to investigate the association of *BADH1* haplotypes with accessions used in the experiment. In the result

77 InDels and 116 SNPs were found in the region of BADH1 gene which represented 39 haplotypes. The findings suggested the usefulness of BADH1 in future experiments based on gene expressions (Min et al., 2021). Some other similar studies are mentioned in Table 10.2.

**TABLE 10.2** Application of NGS on Plants in Response to Environmental Stress

Plant	Study Done	Outcomes	References
<i>Raphanus sativus</i>	To study novel heat-stress related microRNAs	25 miRNAs were identified involved in multiple biological pathways and response to stress	Wang et al. (2015)
<i>Raphanus sativus</i>	Transcriptome profiling to identify genes related to lead stress response	Potential differentially expressed genes involved in response to Pb were identified and their role in different biological pathways was reflected.	Wang et al. (2013)
<i>Raphanus sativus</i>	Transcriptome based gene identification in response to chromium stress.	Genes were identified involved in TFs, metal transporter, antioxidant activity, etc.	Xie et al. (2015)
Tomato	Gene for resistance against tomato late blight disease	Two genes were identified as potential genes for disease resistance	Arafa et al. (2017)
Rice	Analysis of BADH1	Resulted in association of the haplotype BADH1 with salt tolerance	Min et al. (2021)

## 10.7 CONCLUSION

The increasing human population has led to the change in the climate at extreme levels which is affecting the environment. The crop productivity is decreasing due to lesser adaptability of plants to the changing climate. Therefore, the mechanisms that represent the adaptive capacity of any plant are to be understood. Mining of genes and their functions becomes an imperative need to build better cropping systems. This will help in breeding of climate resilient crops by manipulating genes in wild types. The native plants may represent a good source of novel genes that show better adaptability in extreme stress conditions. GWAS and NGS together are accelerating the availability of genetic information of different aspects of any plant, including stress resistance. The available information of loci and QTLs associated with any trait can be used in bioinformatics to enhance

the scope of crop improvement as well. The integrated approaches used are contributing to the development of critical knowledge about the genetic structure of the plants, which are used to develop genetically improved crops to meet the demands of a healthy society. The application of these techniques has proven to be useful in the past years and will provide better opportunities in the coming future.

## ACKNOWLEDGMENT

We are grateful to Director, Dayalbagh Educational Institute and Head, Department of Botany for kind support and encouragement.

## KEYWORDS

- **environmental stress**
- **genome-wide association study**
- **genomics**
- **linkage disequilibrium**
- **mixed liner model**
- **next generation sequencing**
- **transcriptomes**

## REFERENCES

- Arafa, R. A., Rakha, M. T., Soliman, N. E. K., Moussa, O. M., Kamel, S. M., & Shirasawa, K., (2017). Rapid identification of candidate genes for resistance to tomato late blight disease using next-generation sequencing technologies. *PLoS One*, 12, e 0189951.
- Aranzana, M. J., Kim, S., Zhao, K., Bakker, E., Horton, M., Jakob, K., Lister, C., et al., (2005). Genome-wide association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. *PLoS Genet.*, 1, e60.
- Asthir, B., Spoor, W., & Duffus, C. M., (2004). Involvement of polyamines, diamine oxidase and polyamine oxidase in resistance of barley to *Blumeria graminis* F. Sp. hordei. *Euphytica*, 136, 307–312.
- Atkinson, N. J., & Urwin, P. E., (2012). The interaction of plant biotic and abiotic stresses: From genes to the field. *J. Exp. Bot.*, 63, 3523–3543.

- Atwell, S., Huang, Y. S., Vilhjálmsson, B. J., Willems, G., Horton, M., Li, Y., Meng, D., et al., (2010). Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*, 465, 627–631.
- Bandillo, N., Raghavan, C., Muyco, P. A., Sevilla, M. A. L., Lobina, I. T., Dilla-Ermita, C. J., Tung, C. W., et al., (2013). Multi-parent advanced generation inter-cross (MAGIC) populations in rice: Progress and potential for genetics research and breeding. *Rice*, 6, 1–15.
- Bartoli, C., & Roux, F., (2017). Genome-wide association studies in plant pathosystems: Toward an ecological genomics approach. *Front. Plant Sci.*, 8, 763.
- Bhatta, M., Morgounov, A., Belamkar, V., Wegulo, S. N., Dababat, A. A., Erginbas-Orakci, G., Bouhssini, M. E., et al., (2019). Genome-wide association study for multiple biotic stress resistance in synthetic hexaploid wheat. *Int. J. Mol. Sci.*, 20, 3667.
- Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S., (2007). TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics*, 23, 2633–2635.
- Breckle, S. W., (2002). Salinity, halophytes and salt affected natural ecosystems. In: *Salinity: Environment-Plants-Molecules*, 53–77.
- Brown, T. B., Cheng, R., Sirault, X. R., Rungrat, T., Murray, K. D., Trtilek, M., Furbank, R. T., et al., (2014). Trait capture: Genomic and environment modeling of plant phenomic data. *Curr. Opin. Plant Biol.*, 18, 73–79.
- Buckler, E. S., Holland, J. B., Bradbury, P. J., Acharya, C. B., Brown, P. J., Browne, C., Ersoz, E., et al., (2009). The genetic architecture of maize flowering time. *Science*, 325, 714–718.
- Chen, J., Chopra, R., Hayes, C., Morris, G., Marla, S., Burke, J., Xin, Z., & Burow, G., (2017). Genome-wide association study of developing leaves' heat tolerance during vegetative growth stages in a sorghum association panel. *Plant Genom.*, 10, 1–15.
- Church, G. M., (2006). Genomes for all. *Sci. Amer.*, 294, 46–55.
- Daneshmand, F., Arvin, M. J., & Kalantari, K. M., (2010). Physiological responses to NaCl stress in three wild species of potato in vitro. *ActaPhysiol.Plant.*, 32, 91–101.
- Fan, X. D., Wang, J. Q., Yang, N., Dong, Y. Y., Liu, L., Wang, F. W., Wang, N., et al., (2013). Gene expression profiling of soybean leaves and roots under salt, saline-alkali and drought stress by high-throughput Illumina sequencing. *Gene*, 512, 392–402.
- Flint-Garcia, S. A., Thuillet, A. C., Yu, J., Pressoir, G., Romero, S. M., Mitchell, S. E., Doebley, J., et al., (2005). Maize association population: A high-resolution platform for quantitative trait locus dissection. *Plant J.*, 44, 1054–1064.
- Guo, J., Li, C., Zhang, X., Li, Y., Zhang, D., Shi, Y., Song, Y., et al., (2020). Transcriptome and GWAS analyses reveal candidate genes for seminal root length of maize seedlings under drought stress. *Plant Sci.*, 292, 110380.
- Hiersche, M., Rühle, F., & Stoll, M., (2013). Postgwas: Advanced GWAS interpretation in R. *PloS One*, 8, e71775.
- Huang, X., Kurata, N., Wang, Z. X., Wang, A., Zhao, Q., Zhao, Y., Liu, K., et al., (2012). A map of rice genome variation reveals the origin of cultivated rice. *Nature*, 490, 497–501.
- Jeong, S., Kim, J. Y., & Kim, N., (2020). GMStool: GWAS-based marker selection tool for genomic prediction from genomic data. *Sci. Rep.*, 10, 1–12.
- Jin-Long, G., Li-Ping, X., Jing-Ping, F., Ya-Chun, S., Hua-Ying, F., You-Xiong, Q., & Jing-Sheng, X., (2012). A novel dirigent protein gene with highly stem-specific expression from sugarcane, response to drought, salt and oxidative stresses. *Plant Cell Rep.*, 31, 1801–1812.

- Kang, H. M., Sul, J. H., Service, S. K., Zaitlen, N. A., Kong, S. Y., Freimer, N. B., Sabatti, C., & Eskin, E., (2010). Variance component model to account for sample structure in genome-wide association studies. *Nat. Genet.*, 42, 348–354.
- Korte, A., & Farlow, A., (2013). The advantages and limitations of trait analysis with GWAS: A review. *Plant Meth.*, 9, 29.
- Kumar, V., Singh, A., Mithra, S. A., Krishnamurthy, S. L., Parida, S. K., Jain, S., Tiwari, K. K., et al., (2015). Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). *DNA Res.*, 22, 133–145.
- Kump, K. L., Bradbury, P. J., Wissner, R. J., Buckler, E. S., Belcher, A. R., Oropeza-Rosas, M. A., Zwonitzer, J. C., et al., (2011). Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nat. Genet.*, 43, 163–168.
- Lehnert, H., Serfling, A., Friedt, W., & Ordon, F., (2018). Genome-wide association studies reveal genomic regions associated with the response of wheat (*Triticum aestivum* L.) to mycorrhizae under drought stress conditions. *Front. Plant Sci.*, 9, 1728.
- Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., Gore, M. A., et al., (2012). GAPIT: Genome association and prediction integrated tool. *Bioinformatics*, 28, 2397–2399.
- Lippert, C., Listgarten, J., Liu, Y., Kadie, C. M., Davidson, R. I., & Heckerman, D., (2011). FaST linear mixed models for genome-wide association studies. *Nat. Meth.*, 8, 833–835.
- Liu, X. P., & Yu, L. X., (2017). Genome-wide association mapping of loci associated with plant growth and forage production under salt stress in alfalfa (*Medicago sativa* L.). *Front. Plant Sci.*, 8, 853.
- Luo, Z., Szczepanek, A., & Abdel-Haleem, H., (2020). Genome-wide association study (GWAS) analysis of camelina seedling germination under salt stress condition. *Agronomy*, 10, 1444.
- Malosetti, M., Van, D. L. C. G., Vosman, B., & Van, E. F. A., (2007). A mixed-model approach to association mapping using pedigree information with an illustration of resistance to *Phytophthora infestans* in potato. *Genetics*, 175, 879–889.
- Manolio, T. A., (2010). Genome wide association studies and assessment of the risk of disease. *N. Engl. J. Med.*, 363, 166–176.
- Milner, S. G., Jost, M., Taketa, S., Mazón, E. R., Himmelbach, A., Oppermann, M., Weise, S., et al., (2019). Genebank genomics highlights the diversity of a global barley collection. *Nat. Genet.*, 51, 319–326.
- Min, M. H., Maung, T. Z., Cao, Y., Phitaktansakul, R., Lee, G. S., Chu, S. H., Kim, K. W., & Park, Y. J., (2021). Haplotype analysis of BADH1 by next-generation sequencing reveals association with salt tolerance in rice during domestication. *Int. J. Mol. Sci.*, 22, 7578.
- Molina, C., Zaman-Allah, M., Khan, F., Fatnassi, N., Horres, R., Rotter, B., Steinhauer, D., et al., (2011). The salt-responsive transcriptome of chickpea roots and nodules via deep super sage. *BMC Plant Biol.*, 11, 1–26.
- Morison, J. I. L., & Lawlor, D. W., (1999). Interactions between increasing CO<sub>2</sub> concentration and temperature on plant growth. *Plant, Cell Environ.*, 22, 659–682.
- Morkunas, I., Woźniak, A., Mai, V. C., Rucińska-Sobkowiak, R., & Jeandet, P., (2018). The role of heavy metals in plant response to biotic stress. *Molecules*, 23, 2320.
- Myles, S., Peiffer, J., Brown, P. J., Ersoz, E. S., Zhang, Z., Costich, D. E., & Buckler, E. S., (2009). Association mapping: Critical considerations shift from genotyping to experimental design. *The Plant Cell*, 21, 2194–2202.

- Nagel, M., Alqudah, A. M., Bailly, M., Rajjou, L., Pistrick, S., Matzig, G., Börner, A., & Kranner, I., (2019). Novel loci and a role for nitric oxide for seed dormancy and preharvest sprouting in barley. *Plant Cell Environ.*, 42, 1318–1327.
- Nayyeripasand, L., Garoosi, G. A., & Ahmadikehah, A., (2021). Genome-wide association study (GWAS) to identify salt-tolerance QTLs carrying novel candidate genes in rice during early vegetative stage. *Rice*, 14, 1–21.
- Neang, S., De Ocampo, M., Egdane, J. A., Platten, J. D., Ismail, A. M., Seki, M., Suzuki, Y., et al., (2020). A GWAS approach to find SNPs associated with salt removal in rice leaf sheath. *Ann. Bot.*, 126, 1193–1202.
- Oladzad, A., Porch, T., Rosas, J. C., Moghaddam, S. M., Beaver, J., Beebe, S. E., BurrIDGE, J., et al., (2019). Single and multi-trait GWAS identify genetic factors associated with production traits in common bean under abiotic stress environments. *G3: Genes, Genomes, Genetics*, 9, 1881–1892.
- Pantaliao, G. F., Narciso, M., Guimarães, C., Castro, A., Colombari, J. M., Breseghello, F., Rodrigues, L., et al., (2016). Genome-wide association study (GWAS) for grain yield in rice cultivated under water deficit. *Genetica*, 144, 651–664.
- Pariasca-Tanaka, J., Baertschi, C., & Wissuwa, M., (2020). Identification of loci through genome-wide association studies to improve tolerance to sulfur deficiency in rice. *Front. Plant Sci.*, 10, 1668.
- Pedranzani, H., Racagni, G., Alemano, S., Miersch, O., Ramírez, I., Peña-Cortés, H., Taleisnik, E., et al., (2003). Salt tolerant tomato plants show increased levels of jasmonic acid. *Plant Growth Reg.*, 41, 149–158.
- Piepho, H. P., Möhring, J., Melchinger, A. E., & Büchse, A., (2008). BLUP for phenotypic selection in plant breeding and variety testing. *Euphytica*, 161, 209–228.
- Platt, A., Vilhjálmsson, B. J., & Nordborg, M., (2010). Conditions under which genome-wide association studies will be positively misleading. *Genetics*, 186, 1045–1052.
- Poland, J. A., Bradbury, P. J., Buckler, E. S., & Nelson, R. J., (2011). Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *Proceed. Nat. Acad. Sci.*, 108, 6893–6898.
- Quail, M. A., Kozarewa, I., Smith, F., Scally, A., Stephens, P. J., Durbin, R., Swerdlow, H., & Turner, D. J., (2008). A large genome center's improvements to the Illumina sequencing system. *Nat. Meth.*, 5, 1005–1010.
- Rafalski, J. A., (2010). Association genetics in crop improvement. *Curr. Opin. Plant Biol.*, 13, 174–180.
- Raman, H., Raman, R., Qiu, Y., Yadav, A. S., Sureshkumar, S., Borg, L., Rohan, M., et al., (2019). GWAS hints at pleiotropic roles for FLOWERING LOCUS T in flowering time and yield-related traits in canola. *BMC Genom.*, 20, 1–18.
- Rizzo, J. M., & Buck, M. J., (2012). Key principles and clinical applications of “next-generation” DNA sequencing. *Cancer Prev Res.*, 5, 887–900.
- Ronaghi, M., Karamohamed, S., Pettersson, B., Uhlen, M., & Nyren, P., (1996). Real-time DNA sequencing using detection of pyrophosphate release. *Anal. Biochem.*, 242, 84–89.
- Saade, S., Maurer, A., Shahid, M., Oakey, H., Schmöckel, S. M., Negrão, S., Pillen, K., & Tester, M., (2016). Yield-related salinity tolerance traits identified in a nested association mapping (NAM) population of wild barley. *Sci. Rep.*, 6, 1–9.
- Sallam, A., & Martsch, R., (2015). Association mapping for frost tolerance using multi-parent advanced generation inter-cross (MAGIC) population in faba bean (*Vicia faba L.*). *Genetica*, 143, 501–514.

- Sandhu, S. K., Pal, L., Kaur, J., & Bhatia, D., (2019). Genome wide association studies for yield and its component traits under terminal heat stress in Indian mustard (*Brassica juncea* L.). *Euphytica*, 215, 1–15.
- Scholz, M., Gatzek, S., Sterling, A., Fiehn, O., & Selbig, J., (2004). Metabolite fingerprinting: Detecting biological features by independent component analysis. *Bioinformatics*, 20, 2447–2454.
- Schuster, S. C., (2008). Next-generation sequencing transforms today's biology. *Nature Methods*, 5, 16–18.
- Seneviratne, M., Rajakaruna, N., Rizwan, M., Madawala, H. M. S. P., Ok, Y. S., & Vithanage, M., (2019). Heavy metal-induced oxidative stress on seed germination and seedling development: A critical review. *Environ. Geochem. Health*, 4, 1813–1831.
- Tessmann, E. W., & Van, S. D. A., (2018). GWAS for *Fusarium* head blight related traits in winter wheat (*Triticum aestivum* L.) in an artificially warmed treatment. *Agronomy*, 8, 68.
- Thabet, S. G., Moursi, Y. S., Karam, M. A., Graner, A., & Alqudah, A. M., (2018). Genetic basis of drought tolerance during seed germination in barley. *PLoS One*, 13, e0206682.
- Thrash, A., Tang, J. D., DeOrnellis, M., Peterson, D. G., & Warburton, M. L., (2020). PAST: The pathway association studies tool to infer biological meaning from GWAS datasets. *Plants*, 9, 58.
- Tian, F., Bradbury, P. J., Brown, P. J., Hung, H., Sun, Q., Flint-Garcia, S., Rocheford, T. R., et al., (2011). Genome-wide association study of leaf architecture in the maize nested association mapping population. *Nat. Genet.*, 43, 159–162.
- Torrigiani, P., Rabiti, A. L., Bortolotti, C., Betti, L., Marani, F., Canova, A., & Bagni, N., (1997). Polyamine synthesis and accumulation in the hypersensitive response to TMV in *Nicotiana tabacum*. *New Phytol.*, 135, 467–473.
- Tucker, T., Marra, M., & Friedman, J. M., (2009). Massively parallel sequencing: The next big thing in genetic medicine. *Amer.J. Human Genet.*, 85, 142–154.
- Verma, S., Nizam, S., & Verma, P. K., (2013). Biotic and abiotic stress signaling in plants. In: *Stress Signaling in Plants: Genomics and Proteomics Perspective* (Vol. 1, pp. 25–49). Springer, New York, NY.
- Verslues, P. E., Lasky, J. R., Juenger, T. E., Liu, T. W., & Kumar, M. N., (2014). Genome-wide association mapping combined with reverse genetics identifies new effectors of low water potential-induced proline accumulation in *Arabidopsis*. *Plant Physiol.*, 164, 144–159.
- Vilhjálmsón, B. J., & Nordborg, M., (2013). The nature of confounding in genome-wide association studies. *Nat. Rev. Genet.*, 14, 1, 2.
- Wan, H., Chen, L., Guo, J., Li, Q., Wen, J., Yi, B., Ma, C., Tu, J., Fu, T., & Shen, J., (2017). Genome-wide association study reveals the genetic architecture underlying salt tolerance-related traits in rapeseed (*Brassica napus* L.). *Front. Plant Sci.*, 8, 593.
- Wang, L., Yang, Y., Zhang, S., Che, Z., Yuan, W., & Yu, D., (2020). GWAS reveals two novel loci for photosynthesis-related traits in soybean. *Mol. Genet. Genome*, 295.
- Wang, R., Xu, L., Zhu, X., Zhai, L., Wang, Y., Yu, R., Gong, Y., et al., (2015). Transcriptome-wide characterization of novel and heat-stress-responsive microRNAs in radish (*Raphanus sativus* L.) using next-generation sequencing. *Plant Mol. Biol. Rep.*, 33, 867–880.
- Wang, Y., Xu, L., Chen, Y., Shen, H., Gong, Y., Limera, C., & Liu, L., (2013). Transcriptome profiling of radish (*Raphanus sativus* L.) root and identification of genes involved in response to lead (Pb) stress with next generation sequencing. *PLoS One*, 8, e66539.
- Wellcome Trust Case Control Consortium, (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*, 447, 661.



- Xie, Y., Ye, S., Wang, Y., Xu, L., Zhu, X., Yang, J., Feng, H., et al., (2015). Transcriptome-based gene profiling provides novel insights into the characteristics of radish root response to Cr stress with next-generation sequencing. *Front. Plant Sci.*, 6, 202.
- Xu, S., (2003). Theoretical basis of the Beavis effect. *Genetics*, 165, 2259–2268.
- Xu, Z., & Taylor, J. A., (2009). SNPinfo: Integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic Acids Res.*, 37, W600–W605.
- Yang, Y., Wang, L., Zhang, D., Cheng, H., Wang, Q., Yang, H., & Yu, D., (2020). GWAS identifies two novel loci for photosynthetic traits related to phosphorus efficiency in soybean. *Mole. Breed.*, 40, 1–14.
- Yu, J., Pressoir, G., Briggs, W. H., Bi, I. V., Yamasaki, M., Doebley, J. F., McMullen, M. D., et al., (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.*, 38, 203–208.
- Zhang, Z., Buckler, E. S., Casstevens, T. M., & Bradbury, P. J., (2009). Software engineering the mixed model for genome-wide association studies on large samples. *Brief. Bioinformatics*, 10, 664–675.
- Zhang, Z., Ersoz, E., Lai, C. Q., Todhunter, R. J., Tiwari, H. K., Gore, M. A., Bradbury, P. J., et al., (2010). Mixed linear model approach adapted for genome-wide association studies. *Nat. Genet.*, 42, 355–360.
- Zhou, X., & Stephens, M., (2012). Genome-wide efficient mixed-model analysis for association studies. *Nat. Genet.*, 44, 821–824.
- Zhu, J. K., (2002). Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.*, 53, 247–273.

## CHAPTER 11

---

# CRISPR/Cas and Its Potentiality as an Effective Tool in Understanding Environmental Stress Response

DEEPU PANDITA

*Government Department of School Education, Jammu,  
Jammu and Kashmir, India, E-mail: deepupandita@gmail.com*

---

### ABSTRACT

The potential tools of genome editing revolutionize environmental stress resistance in plants. CRISPR/Cas-mediated genome editing for the designing of stress-tolerant plants is robust, versatile, cheap, specific, and efficient. CRISPR/Cas system has 6 types of I, III, and IV (Class 1), II, V, and VI (Class 2) with different Cas variants and nucleases. CRISPR-associated protein (Cas) endonuclease generates single site-specific DNA double-strand breaks (DSBs). The naturally occurring repairing pathways repair DSBs by high-fidelity homology coordinated repair (HDR) and predominant error-prone non-homologous end joining (NHEJ), which lead to gene knockout or knock-in mutations or precise gene replacement. NHEJ generates deletions or insertions at the DSB site, leading to frameshift and loss in gene function. HDR approach repairs point mutations and accelerates crop improvement. This chapter will focus on the contemporary know-how and prospective applications of CRISPR/Cas mediated plant genome editing to provide insights into the complex traits of environmental stress response.

## 11.1 INTRODUCTION

Improvement of crop traits for designing the resistance against biotic and abiotic stresses is a continual process worldwide from a long duration. Acclimatizing advanced approaches and innovative tools is need of high priority for sustainable and climate-smart food production and reduce effects greatest threat of climate change (Lobell & Gourdji, 2012). Conventional breeding was most effective way of crop trait improvement but is time-consuming (a period of about 8–10 years), backbreaking and costly approach. Molecular marker technology of restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single-nucleotide polymorphism (SNP) and diversity arrays technology (DArT) markers characterized plants under stress scenarios (Rao et al., 2016). This tool may not produce stress-tolerant plants due to complex inheritance and interactions of genotype and environment (Bhat et al., 2016). Further, conventional breeding allows transmission of undesirable genes alongside gene of Interest (GOI) to future progeny of plants. Because of which multiple back-crossing events for a number of generations with recipient is required to eradicate the unpredicted linked traits and to introduce the desired trait from donor. Genetic engineering improved crops which were exposed to any kind of biotic and abiotic stress by recombinant DNA technology. But there are cost, timewasting, efficacies, social, and ethical concerns, and further biosafety protocols limit approval for the genetically modified (GM) crops (Prado et al., 2014; Raman, 2017). In GMOs, only genes of Interest (GOI) are transferred to the recipient plant but there is non-targeted integration. So, need of the hour is any new tool for improvement of the plants subjected to stress which can withstand the upcoming scenarios of global warming and climate changes. Beginning of various advanced genome editing platforms involving molecular techniques has revolutionized the area of agricultural crops. Sequence-specific nucleases (SSNs) recognize precise and specific target DNA sites and introduce double-stranded breaks (DSBs) at specific genomic loci in plants (Zhang et al., 2018; Costa et al., 2017). CRISPR (clustered regularly interspaced short palindromic repeat)/Cas (CRISPR-associated protein) system which is a new breeding technique is simple, robust, prompt, highly efficient, low-cost, precise, adaptable, and broadly acknowledged tool (Zhang et al., 2018). Genome editing takes only 4–6 years wherein precise modification of regulatory region of target genes or target gene itself is done. CRISPR/Cas system consists of a complex of Cas9 endonuclease and single guide RNA (sgRNA). The sgRNA gives

target specificity on DNA strand and Cas9 endonuclease creates a double-stranded break (DSB). DSBs can be repaired by endogenous DNA repair mechanism of cells involving error prone non-homologous end joining (NHEJ) and homologous recombination (HR) or homology directed repair (HDR) (Chen & Gao, 2014). NHEJ creates nucleotide insertions or deletions causing gene knockouts. HDR pathway generates precise base modifications or gene replacement in the presence of donor DNA sequence (Chen & Gao, 2014). In turn novel mutants get generated (Voytas & Gao, 2014). Various reviews and chapters describe CRISPR/Cas genome-editing platform-based relevance in crop improvement (Zhang et al., 2018; Sedeek et al., 2019; Pandita, 2021a–c).

## 11.2 CLASSIFICATION OF CRISPR/Cas SYSTEMS

CRISPR/Cas system, a defensive mechanism for the degradation of foreign genomes was discovered in year 1987 as a set of 29 nucleotide repeats downstream of *iap* gene (Ishino et al., 1987). Almost 44% of 703 archaeal systems own one or more CRISPR/Cas modules in their genomes (Koonin & Makarova, 2009; Deveau et al., 2010). CRISPR/Cas loci involve CRISPR array. CRISPR array has two to several 100 direct, partially palindromic, normally exact repeats (of 25–35 bp each). These repeats are disconnected by inimitable spacers (of 30–40 bp) and end-to-end clusters of multiple *cas* genes organized in one or more operons which encode both the adaptation and effector modules, often with accessory genes (Makarova et al., 2013, 2015). CRISPR/Cas systems are broadly categorized into two major classes: Class 1 and Class 2 on the basis of design principles of the effector modules as well as signature *cas* genes/*cas* loci structure, organization of *cas* operons and phylogeny of conserved Cas proteins. Class 1 systems rely on heteromeric multisubunit effector complexes comprising numerous Cas proteins and class 2 systems in which effector consists of single, large, multi-domain effector protein (Makarova et al., 2015, 2018, 2020). Two CRISPR-Cas classes in the next hierarchical level divide into 6 main Types I, III, and IV for class 1 including 16 subtypes, and types II, V, and VI for class 2 includes 3 types and 17 subtypes (Makarova et al., 2020). Each type has distinctive architecture of effector modules including unique signature proteins. Each of these contains a total of 33 multiple subtypes and multiple variants distinguished by subtler differences in locus organization and often encode subtype-specific Cas proteins (Makarova et al., 2020). Type I, II, and

V targets DNA; type VI targets RNA and type III targets both the DNA and RNA (Samai et al., 2015; Koonin et al., 2017). In several organisms, Cas loci do not fit into current classification due to their complex modular structure and variability, e.g., target of Cas13d is not DNA, but RNA. Class 2 includes Cas9, Cas12, and Cas13 endonucleases which are widely used as genome editing tools (Wu et al., 2018).

### 11.3 MECHANISM OF ACTION OF CRISPR/Cas SYSTEMS

CRISPR/Cas system has a basis from the natural phenomena of bacterial and archaeal adaptive immune system for invasion against external plasmids or sequences of viruses (Marraffini & Sontheimer, 2010). CRISPR/Cas editing system has two components which comprise Cas endonuclease and single guide RNA (sgRNA). Cas-sgRNA complex binds to the target DNA sequences. The sgRNA is made of CRISPR RNA (crRNA) with programmable 20-nt RNA target sequences and trans-activating crRNA (tracrRNA). The sgRNA recognizes and directs Cas protein to specific genomic target (Hsu et al., 2014). Cas induces double-strand break (DSB) at the loci of interest in the genome. The specificity of the system is due to 20 nt elongated replaceable guide sequence within sgRNA, complementary to the target sequence (Li et al., 2013). The most generally used Cas protein is Cas9 endonuclease derived from the gram-positive *Streptococcus pyogenes* bacterium. Cas9 endonuclease cleavage is based on Protospacer Adjacent Motif (PAM, 5'-NGG-3') present directly downstream of 20-nt target DNA sequence (Zhang et al., 2018) and PAM requirement is a bottleneck in it. PAM sequence varies on the basis of type of CRISPR/Cas, e.g., in Cas9 variants and Cas9 orthologs (Leenay & Beisel, 2017). The Cas9 endonuclease cleaves at 3-nt upstream of PAM within DNA target site, generating blunt ended double-strand breaks (DSBs) (Jiang & Doudna, 2017). CRISPR from *Prevotella* and *Francisella* (Cpf1) known as Cas12a endonuclease of class 2 type V lacks HNH domain, needs T rich PAM and generates staggered cuts for the plant genetic manipulations (Zetsche et al., 2015; Stella et al., 2017). DSBs activated intrinsic DNA repair mechanism of the cell through NHEJ or homology-directed repair (HDR) to generate gene knockout or gene knock-in. HDR requires repair donor DNA template and leads to knock-in. NHEJ is most dominant and active error prone DNA repair pathway and generates insertions and deletions (indels) or substitutions resulting in the gene knockout or gene silencing (Rong & Golic, 2000; Hasley et al., 2021).

## 11.4 CRISPR/Cas-MEDIATED ADVANCEMENTS IN PLANT PROTECTION

Plant improvement by gene knockout and replacement of CRISPR/Cas9 has been very efficient. But 5'-NGG-3' PAM sequence specificity has set limitations on usage of CRISPR/Cas9 to prospective targets. Multiple Cas variants having diverse specificity of PAM sequences have been characterized (Wrighton, 2018). SpCas9-NAG and xCas9 find use in *Oryza sativa* (Meng et al., 2018; Hu et al., 2018). CRISPR/Cas12a system has widened the skyline of genome editing. Cas12a (Cpf1) endonuclease was derivative of *Francisella novicida* (FnCpf1) and its ortholog from Lachnospiraceae bacterium (LbCpf1). FnCpf1 and LbCpf1 recognize T-rich (5'-TTTN-3') PAM sequence and generate cohesive (4–5 nucleotide overhangs) ended DSBs. CRISPR/Cas12a system overpowers target limits of system of the CRISPR/Cas9 (Zetsche et al., 2015). The CRISPR/Cas12a has been efficiently used for targeted mutagenesis in *Arabidopsis thaliana*, *Nicotiana tabacum*, *Oryza sativa* L. and *Glycine max* L. (Endo et al., 2016; Tang et al., 2017). The Cas12a variants facilitate multiplex gene editing and recognize TYCV PAM sequences (Li et al., 2018). CRISPR/Cas edited plants show off-target mutations and inefficient ability of regeneration. These are major bottlenecks for CRISPR/Cas system applications in the crop improvement programs. These roadblocks can be overcome by procedure of edited pollens and immature embryos which outdo *in-vitro* tissue culturing and use of stress-inducible CRISPR/Cas methods consequential of trivial off-target activities (Kelliher et al., 2019; Nandy et al., 2019).

### 11.4.1 POTENTIALITY OF CRISPR/Cas-MEDIATED EFFECTIVE TOOL IN UNDERSTANDING ABIOTIC STRESS RESPONSE

For the first time, CRISPR/Cas9 based genome editing was reported in model plants for instance *Arabidopsis thaliana* L., *Nicotiana benthamiana* and *Oryza sativa* L. (Li et al., 2013; Shan et al., 2013; Nekrasov et al., 2013). The CRISPR/Cas9 based manipulation of genes enabled abiotic stress response and generation of stress resilient crops in plant science. Improved expression of ARGOS8 which negatively regulates ethylene responses in maize was achieved by the precise genome editing through CRISPR/Cas9 resulting in enhanced drought tolerance and improved yield (Shi et al., 2017). A tissue-specific AtEF1 promoter derived truncated gRNAs (tru-gRNAs) and Cas9

cause mutations in an abiotic stress-responsive gene named as OST2/AHA1 (Osakabe & Osakabe, 2017). This leads to improved stomatal response in *Arabidopsis thaliana*. OsRR22, OsSIT1, and OsNAC041 genes increased salinity tolerance in Rice (Li et al., 2014; Zhang et al., 2019; Bo et al., 2019). CRISPR/Cas9 facilitated editing of cold tolerance MYB30 gene and panicle length *OsPIN5b* gene and grain size *GS3* gene (Zeng et al., 2020), OsCOLD1 (Ma et al., 2015), and TIFY1a, TIFY1b (Huang et al., 2017) genes improved cold tolerance in *Oryza sativa* L. Similarly, CRISPR/Cas9 mediated editing of NPRI (Erpen-Dalla et al., 2019), SINPR1 (knock-out) (Li et al., 2019) and SIMAPK3 (knock-out) (Wang et al., 2017a) improved tolerance to water deficiency in tomato plants. CRISPR/Cas9 mediated editing of Heat Shock Protein 90 (HSP90) increased tolerance to high temperature in *Tetraselmis suecica* (Xu et al., 2020). CRISPR/Cas9 based editing of Drb2a and Drb2b increased salinity and drought tolerance whereas SAPK1 and SAPK2 enhanced salt tolerance in *Glycine max* L. (Curtin et al., 2018; Lou et al., 2017). Cpf1 has been used in *Arabidopsis thaliana* L. and *Oryza sativa* L. (Tang et al., 2017, 2018). Other examples of CRISPR/Cas based genome editing approaches with promise in fighting devastating abiotic plant stress conditions are summarized in Table 11.1.

#### **11.4.2 POTENTIALITY OF CRISPR/Cas-MEDIATED EFFECTIVE TOOL IN UNDERSTANDING BIOTIC STRESS RESPONSE**

CRISPR/Cas mediated genome editing produced tolerance in plants towards viruses, bacteria, nematodes, insects, and fungal pathogens. Editing of recessive eIF4E gene increased resistance to Papaya ring-spot mosaic virus-W, Zucchini yellow mosaic virus and cucumber vein yellowing virus in *Cucumis sativus* L. (Chandrasekaran et al., 2016). CRISPR/Cas9 based targeting of Initiation factors like eIF4E and eIF(iso) 4E eIF4E/exon involved in infection of Turnip mosaic virus (TuMV) in *Arabidopsis thaliana* L., and eIF4G involved in infection of Rice Tungro Disease Resistance developed resistance in these plants (Pyott et al., 2016; Macovei et al., 2018). CRISPR/Cas9-mediated targeting of *Ocimum basilicum* candidate susceptibility gene ObDMR6 which is a homolog of DMR6 generated transgene-free downy mildew-resistant sweet basil varieties (Hasley et al., 2021). CRISPR/Cas9 generated transgene-free mutants in sweet basil by *Agrobacterium*-mediated transformation (Navet & Tian, 2020). CRISPR/Cas inactivated endogenous banana streak provirus in banana (Tripathi et al.,

**TABLE 11.1** Plant Abiotic Stress Conditions Improved by CRISPR/Cas

Plant Species	Target Gene	Target Traits Improved	References
CRISPR/Cas9			
<i>Carica papaya</i> L.	CpDreb2	Resistance to drought, heat and cold	Arroyo-Herrera et al. (2016)
<i>Carica papaya</i> L.	CpRap2.4a and CpRap2.4b	Resistance to heat and cold	Figueroa-Yanez et al. (2016)
<i>Gossypium</i> spp.	GhPIN1-3 and GhPIN2	Resistance to drought	He et al. (2017)
<i>Gossypium</i> spp.	GhRDL1	Resistance to drought	Dass et al. (2017)
<i>Hordeum vulgare</i> L.	ALMT	High Al <sup>3+</sup>	Zhou et al. (2014)
<i>Manihot esculenta</i> Crantz	MeKUPs	Resistance to salt, osmosis, cold, drought	Ou et al. (2018)
<i>Manihot esculenta</i> Crantz	MeMAPKKK	Resistance to drought	Ye et al. (2017)
<i>Musa acuminata</i> Colla	MaSWEET-4d, MaSWEET-1b, and MaSWEET-4b	Resistance to cold and salt	Miao et al. (2017a)
<i>Musa acuminata</i> Colla	MaAPS1 AND MaAPL3	Resistance to cold and salt	Miao et al. (2017b)
<i>Oryza sativa</i> L.	Sub1A, SK1 and SK2	Resistance to flooding	Xu et al. (2006); Fukao et al. (2006); Hattori et al. (2009)
<i>Oryza sativa</i> L.	DRO1	Resistance to drought	Uga et al. (2011, 2013)
<i>Oryza sativa</i> L.	NRAT1	High Al <sup>3+</sup>	Li et al. (2014)
<i>Oryza sativa</i> L.	PSTOL1 at the Pup1 locus	Low Pi	Gamuyao et al. (2012); Chin et al. (2011)
<i>Oryza sativa</i> L.	<i>Oryza sativa</i> phosphate transporter 4 (OsPT4)	Absence of OsPT4 activity reduces arsenic uptake from soil in rice plant	Ye et al. (2017)
<i>Oryza sativa</i> L.	Natural resistance associated macrophage proteins 5 (OsNRAMP5) of <i>Oryza sativa</i>	Absence of OsNRAMP5 reduce the uptake of cadmium from soil	Tang et al. (2017)
<i>Oryza sativa</i> L.	Arsenite-responsive MYB1 of <i>Oryza sativa</i> (OsARM1)	Plant lacking the activity of OsARM1 show tolerance to as stress	Wang et al. (2017)



**TABLE 11.1** (Continued)

Plant Species	Target Gene	Target Traits Improved	References
<i>Oryza sativa</i> L.	OsHAK1	Inactivation of OsHAK1 reduces the uptake of 137Cs from soil	Nieves-Cordones et al. (2017)
<i>Phoenix dactylifera</i> L.	Pdpcs and Pdmt	Cadmium and chromium toxicity	Chaabene et al. (2018)
<i>Phoenix dactylifera</i> L.	Pdpcs and Pdmt	Heavy metal toxicity	Chaabene et al. (2018)
<i>Saccharum officinarum</i> L.	ScAPX6	Resistance to copper	Liu et al. (2017)
<i>Saccharum officinarum</i> L.	ScNsLTP	Resistance to drought and chilling	Chen et al. (2017)
<i>Solanum lycopersicum</i> L.	Mitogen-activated protein kinase 3 (S1MAPK3) in <i>Solanum lycopersicum</i>	Absence of S1MAPK3 activity make plant susceptible to drought stress	Wang et al. (2017b)
<i>Triticum aestivum</i> L. and <i>Hordeum vulgare</i> L.	VRN1 at the FR1 locus and CBFs at the FR2 locus	Resistance to Low temperature	Dhillon et al. (2012); Stockinger et al. (2007); Kinoo et al. (2010); Francia et al. (2007)
<i>Triticum aestivum</i> L. and <i>Oryza sativa</i> L.	TaHKT1;5	Saline soil	Dubcovsky et al. (1996)
<i>Zea mays</i> L. CRISPR/Cas 12	MATE1	High Al <sup>3+</sup>	Maron et al. (2013)
<i>Oryza sativa</i> L.	OsEPFL9	Regulated stomatal density in rice leaves	Yin et al. (2019)
<i>Oryza sativa</i> L.	OsDEP1, OsPDS, and OsEPFL9	Abiotic stress tolerance	Zhong et al. (2018)
<i>Solanum lycopersicum</i> L.	HKT1;2 HDR	Multiple-stress tolerance	Vu et al. (2020)

2019). The promoters of OsSWEET11 and OsSWEET14, and OsSWEET13 on targeting with CRISPR/Cas9 enabled bacterial blight susceptibility and bacterial blight tolerance in *Oryza sativa* L., respectively (Jiang et al., 2013; Zhou et al., 2015; Oliva et al., 2019; Zafar et al., 2020). Resistance to cotton leaf curl Kokhran virus (CLCuKoV) and begomoviruses can be produced by CRISPR/Cas9 based editing of conserved nonanucleotide sequences (TYLCV, BCTV-Worland, CLCuKoV, MeMV, TYLCSV, and BCTV-Logan) in *Nicotiana benthamiana* (Ali et al., 2016; Zaidi et al., 2016). CRISPR/Cas9 genome-edited targeting of Gh14-3-3 enabled tolerance to cotton verticillium wilt (Zhang et al., 2018). CRISPR/Cas9 targeting of coat protein (CP) or replicase (Rep) resulted in tomato plants resistant to TYLCV (Tashkandi et al., 2018) and SIJAZ2 targeting enabled speck resistance in tomato (Ortigosa et al., 2019). Cucumber mosaic virus (CMV) or tobacco mosaic virus (TMV) were targeted by CRISPR/Cas through *Francisella novicida* Cas9 (FnCas9) in *Nicotiana benthamiana* and *Arabidopsis thaliana* L. (Zhang et al., 2018). Gene-edited knock out of MLO (Mildew Locus O) susceptibility (S) gene homologs renders powdery mildew resistance due to *Blumeria graminis* f. sp. *tritici* (Bgt) in wheat (Wang et al., 2014). CRISPR/Cas9 edited MLO genes in tomato plants namely “tomelo” are powdery mildew resistant (Nekrasov et al., 2017). MLO7 targeting in grape controlled Erysiphe necator which causes powdery mildew in grape (*Vitis vinifera* L.) cultivar Chardonnay (Pessina et al., 2016) and WRKY52 targeting enabled resistance to the gray mold in *Vitis vinifera* L. (Wang et al., 2018). CRISPR/Cas9 targeted, DIPM-1, DIPM-2 and DIPM-4 approach developed *Malus domestica* Borkh. plants with resistance to fire blight enterobacterial pathogen *Erwinia amylovora* (Malnoy et al., 2016). CRISPR/Cas9 based genome editing developed blast resistant *Oryza sativa* L. japonica through target of codons near translation initiation codon of OsERF922 with sgRNA to introduce insertions and deletions (Wang et al., 2016). LwaCas13a from *Leptotrichia wadei* and PspCas13b from *Prevotella* sp. were widely used in *Oryza sativa* (Abudayyeh et al., 2016; Cox et al., 2017). CRISPR/Cas9 mediated editing of nCBP-1 & nCBP-2/exon, Non-Expressor of Pathogenesis-Related 3 (TcNPR3) gene, and WRKY70, WRKY11 enabled brown streak (RNA) in *Manihot esculenta* Crantz (Gomez et al., 2019), Black pod disease in Cacao (Fister et al., 2018), and JA-induced and SA-induced tolerance to pathogens in *Brassica napus* subsp. *napus* (Sun et al., 2018). Other examples of CRISPR/Cas based genome editing approaches with promise in fighting devastating biotic plant diseases are précised in Table 11.2.

**TABLE 11.2** Plant Biotic Stress Conditions Improved by CRISPR/Cas

Plant Species	Target Gene	Target Traits Improved	References
CRISPR/Cas9			
<i>Citrus × paradisi</i>	CsLOB1 promoter	Alleviated citrus canker	Jia et al. (2016)
<i>Citrus × paradisi</i>	CsLOB1	Citrus canker resistance	Jia et al. (2017)
<i>Cucumis sativus</i>	eIF4E	Virus resistance cucumber vein yellowing virus	Chandrasekaran et al. (2016)
<i>Malus domestica</i> Borkh.	DIPM-1, DIPM-2, and DIPM-4	Resistance to fire blight disease	Malnoy et al. (2016)
<i>Ocimum basilicum</i> L.	ObDMR6	Downy mildew resistance	Hasley et al. (2021)
Orange	CsLOB1 promoter	Citrus canker resistance	Peng et al. (2017)
<i>Oryza sativa</i> L.	OsERF922	Enhanced rice blast resistance	Wang et al. (2016)
<i>Oryza sativa</i> L.	OsSWEET13 SWEET	Bacterial blight resistance by <i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Zhou et al. (2015); Oliva et al. (2019)
<i>Oryza sativa</i> L.	ALS	Resistance to herbicides	Endo et al. (2016)
<i>Oryza sativa</i> L.	ALS	Resistance to herbicides	Sun et al. (2016)
<i>Oryza sativa</i> L.	EPSPS	Resistance to herbicides	Li et al. (2016)
<i>Oryza sativa</i> L.	ALS	Resistance to herbicides	Butt et al. (2017)
<i>Solanum lycopersicum</i> L.	S1MLO1	Powdery mildew resistance	Nekrasov et al. (2017)
<i>Solanum lycopersicum</i> L.	S1JAZ2	Bacterial speck resistance	Ortigosa et al. (2018)
<i>Solanum lycopersicum</i> L.	DMR6 KO	Disease resistance against <i>Pseudomonas syringae</i> pv. tomato (Pto), <i>Phytophthora capsica</i> and <i>Xanthomonas</i> spp.	De Toledo Thomazella et al. (2016)
<i>Solanum tuberosum</i> L.	DMR6	Downy mildew resistance	Sun et al. (2016)
<i>Triticum aestivum</i> L.	EDR1	Powdery mildew resistance	Zhang et al. (2017)
<i>Triticum aestivum</i> L.	Six MLO alleles	<i>Blumeria graminis</i> f. sp. <i>tritici</i> . resistance	Wang et al. (2014)
CRISPR/Cas 13			
<i>Nicotiana benthamiana</i>	HC-Pro and GFP2 genes	Resistance to turnip mosaic virus (TuMV)	Aman et al. (2018a)
<i>Arabidopsis thaliana</i>	TuMV virus	Resistance to TuMV virus	Aman et al. (2018b)

## 11.5 CONCLUSION

The global warming and climatic changes accompanied with various types of spontaneous and dynamic environmental stresses are the highest threat to food security. Conventional breeding and genetic engineering tactics have given substantial gifts for the mitigation of these stresses. These tools are not competent enough to design desirable alleles in an in-effect mode in a short time. CRISPR/Cas based editing tools generate accurate and precise modifications in a simple way, with no time and transform the breeding programs to designing of climate-smart crops.

## KEYWORDS

- **Cas endonuclease**
- **CRISPR/Cas**
- **crop improvement**
- **environmental stress**
- **genome editing**
- **genome engineering**

## REFERENCES

- Abudayyeh, O. O., Jonathan, S., Gootenberg, K. S., Joung, J., Ian, M. S., David, B. T. C., & Shmakov, S., (2016). C2c2 is a single component programmable RNA guided RNA-targeting CRISPR effector. *Science*, 353(6299): <https://doi.org/10.1126/science.aaf5573>.
- Ali, Z., Ali, S., Tashkandi, M., Zaidi, S., & Mahfouz, M., (2016). CRISPR/Cas9-mediated immunity to Gemini viruses: Differential interference and evasion. *Sci. Rep.*, 6, 26912.
- Aman, R., Ali, Z., Butt, H., et al., (2018a). RNA virus interference via CRISPR/Cas13a system in plants. *Genome Biol.*, 19(1), 1–9. <https://doi.org/10.1186/s13059-017-1381-1>.
- Aman, R., Mahas, A., Butt, H., Aljedaani, F., & Mahfouz, M., (2018b). Engineering RNA virus interference via the CRISPR/Cas13 machinery in *Arabidopsis*. *Viruses*, 10(12), 732. <https://doi.org/10.3390/v10120732>.
- Arroyo-Herrera, A., Figueroa-Yáñez, L., Castaño, E., Santamaría, J., Pereira-Santana, A., Espadas-Alcocer, J., & Sánchez-Teyer, F., (2016). A novel Dreb2-type gene from *Carica papaya* confers tolerance under abiotic stress. *Plant Cell, Tissue and Organ Culture*, 125(1), 119–133. doi: 10.1007/s11240-015-0934-9.

- Bhat, J. A., Ali, S., Salgotra, R. K., Mir, Z. A., Dutta, S., Jadon, V., Tyagi, A., et al., (2016). Genomic selection in the era of next generation sequencing for complex traits in plant breeding. *Frontiers in Genetics*, 7, 221.
- Bo, W., Zhaohui, Z., Huanhuan, Z., Xia, W., Binglin, L., Lijia, Y., Xiangyan, H., et al., (2019). Targeted mutagenesis of NAC transcription factor gene, OsNAC041, leading to salt sensitivity in rice. *Rice Sci.*, 26, 98–108.
- Butt, H., Eid, A., Ali, Z., Atia, M. A. M., Mokhtar, M. M., Hassan, N., et al., (2017). Efficient CRISPR/Cas9-mediated genome editing using a chimeric single-guide RNA molecule. *Front Plant Sci.*, 8, 1441.
- Chaâbene, Z., Rorat, A., Hakim, I., Bernard, F., Douglas, G. C., Elleuch, A., et al., (2018). Insight into the expression variation of metal-responsive genes in the seedling of date palm (*Phoenix dactylifera*). *Chemosphere*, 197, 123–134. doi: 10.1016/j.chemosphere.2017.12.146.
- Chandrasekaran, J., Brumin, M., Wolf, D., Leibman, D., Klap, C., Pearlsman, M., et al., (2016). Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Mol. Plant Pathol.*, 17, 1140–1153.
- Chen, K., & Gao, C., (2014). Targeted genome modification technologies and their applications in crop improvements. *Plant Cell Rep.*, 33, 575–583. <https://doi.org/10.1007/s00299-013-1539-6>.
- Chen, Y., Ma, J., Zhang, X., et al., (2017). A novel non-specific lipid transfer protein gene from sugarcane (NsLTPs), obviously responded to abiotic stresses and signaling molecules of SA and MeJA. *Sugar Technology*, 19(1), 17. doi: 10.1007/s12355-016-0431-4.
- Chin, J. H., Gamuyao, R., Dalid, C., Bustamam, M., Prasetyono, J., Moeljopawiro, S., Wissuwa, M., & Heuer, S., (2011). Developing rice with high yield under phosphorus deficiency: Pup1 sequence to application. *Plant Physiol.*, 156(3), 1202–1216. doi: 10.1104/pp.111.175471.
- Costa, J. R., Bejcek, B. E., McGee, J. E., Fogel, A. I., Brimacombe, K. R., & Ketteler, R., (2017). Genome editing using engineered nucleases and their use in genomic screening. In: Sittampalam, G. S., Grossman, A., Brimacombe, K., et al., (eds.), *Assay Guidance Manual*. Eli Lilly & Company and the National Center for Advancing Translational Sciences, Bethesda.
- Cox, D. B. T., Gootenberg, J. S., Abudayyeh, O. O., Franklin, B., Kellner, M. J., Joung, J., & Zhang, F., (2017). RNA editing with CRISPR-Cas13. *Science*, 358(6366), 1019–1027. doi: 10.1126/science.aag0180.
- Curtin, S. J., Xiong, Y., Michno, J. M., Campbell, B. W., Stec, A. O., Ćermák, T., Starker, C., et al., (2018). CRISPR/Cas9 and TALENs generate heritable mutations for genes involved in small RNA processing of *Glycine max* and *Medicago truncatula*. *Plant Biotechnol. J.*, 16, 1125–1137.
- Dass, A., Abdin, M. Z., Reddy, V. S., & Leelavathi, S., (2017). Isolation and characterization of the dehydration stress-inducible GhRDL1 promoter from the cultivated upland cotton (*Gossypium hirsutum*). *Journal of Plant Biochemistry and Biotechnology*, 26(1), 113–119. doi: 10.1007/s13562-016-0369-3.
- De Toledo, T. D. P., Brail, Q., Dahlbeck, D., & Staskawicz, B. J., (2016). *CRISPR-Cas9 Mediated Mutagenesis of a DMR6 Ortholog in Tomato Confers Broad-Spectrum Disease Resistance*. Pre-print at BioRxiv <http://dx.doi.org/10.1101/064824>.
- Deveau, H., Garneau, J. E., & Moineau, S., (2010). CRISPR/Cas system and its role in phage-bacteria interactions. *Annual Review of Microbiology*, 64, 475–493.

- Dhillon, T., Pearce, S. P., Stockinger, E. J., Distelfeld, A., Li, C., Knox, A. K., et al., (2012). Regulation of freezing tolerance and flowering in temperate cereals: The VRN-1 connection. *Plant Physiology*, 153(4), 1846–1858. doi: [org/10.1104/pp.110.159079](https://doi.org/10.1104/pp.110.159079).
- Dubcovsky, J., María, G. S., Epstein, E., Luo, M. C., & Dvořák, J., (1996). Mapping of the  $K^+/Na^+$  discrimination locus Kna1 in wheat. *Theoretical and Applied Genetics*, 92(3, 4), 448–454. doi: [10.1007/BF00223692](https://doi.org/10.1007/BF00223692).
- Endo, A., Masafumi, M., Kaya, H., & Toki, S., (2016). Efficient targeted mutagenesis of rice and tobacco genomes using Cpf1 from *Francisella novicida*. *Sci. Rep.*, 6, 38169.
- Endo, M., Mikami, M., & Toki, S., (2016). Biallelic gene targeting in rice. *Plant Physiol.*, 170, 667–677.
- Erpen-Dalla, C. L., Mahmoud, L. M., Moraes, T. S., Mou, Z., Grosser, J. W., & Dutt, M., (2019). Development of improved fruit, vegetable, and ornamental crops using the CRISPR/cas9 genome editing technique. *Plants*, 8, 601.
- Figueroa-Yañez, L., Pereira-Santana, A., Arroyo-Herrera, A., Rodriguez-Corona, U., Sanchez-Teyer, F., EspadasAlcocer, J., et al., (2016). RAP2.4a is transported through the phloem to regulate cold and heat tolerance in papaya tree (*Carica papaya* cv. Maradol): Implications for protection against abiotic stress. *PLoS One*, 11(10), 0165030. doi: [10.1371/journal.pone.0165030](https://doi.org/10.1371/journal.pone.0165030).
- Francia, E., Barabaschi, D., Tondelli, A., Laidò, G., Rizza, F., Stanca, A. M., Busconi, M., et al., (2007). Fine mapping of a HvCBF gene cluster at the frost resistance locus Fr-H2 in barley. *Theoretical and Applied Genetics*, 115(8), 1083–1091. doi: [10.1007/s00122-007-0634-x](https://doi.org/10.1007/s00122-007-0634-x).
- Fukao, T., Xu, K., Ronald, P. C., & Bailey-Serres, J., (2006). A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *The Plant Cell*, 18, 2021–2034. doi: [10.1105/tpc.106.043000](https://doi.org/10.1105/tpc.106.043000).
- Gamuyao, R., Chin, J. H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., et al., (2012). The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature*, 488(7412), 535–539. doi: [10.1038/nature11346](https://doi.org/10.1038/nature11346).
- Gomez, M. A., Lin, Z. D., Moll, T., Chauhan, R. D., Hayden, L., Renninger, K., Beyene, G., Taylor, N. J., Carrington, J. C., Staskawicz, B. J., et al., (2019). Simultaneous CRISPR/Cas9-mediated editing of cassava eIF4E isoforms nCBP-1 and nCBP-2 reduces cassava brown streak disease symptom severity and incidence. *Plant Biotechnol. J.*, 17(2), 421–434. doi: [10.1111/pbi.12987](https://doi.org/10.1111/pbi.12987).
- Hasley, J. A. R., Navet, N., & Tian, M., (2021). CRISPR/Cas9-mediated mutagenesis of sweet basil candidate susceptibility gene ObDMR6 enhances downy mildew resistance. *PLoS One*, 16(6), e0253245. <https://doi.org/10.1371/journal.pone.0253245>.
- Hattori, Y., Nagai, K., Furukawa, S., Song, X. J., Kawano, R., Sakakibara, H., Wu, J., et al., (2009). The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature*, 460(7258), 1026–1030. doi: [10.1038/nature08258](https://doi.org/10.1038/nature08258).
- He, P., Zhao, P., Wang, L., Zhang, Y., Wang, X., Xiao, H., et al., (2017). The PIN gene family in cotton (*Gossypium hirsutum*): Genome-wide identification and gene expression analyses during root development and abiotic stress responses. *BMC Genomics*, 18, 507. doi: [10.1186/s12864-017-3901-5](https://doi.org/10.1186/s12864-017-3901-5).
- Hsu, P. D., Lander, E. S., & Zhang, F., (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, 157, 1262–1278. <https://doi.org/10.1016/j.cell.2014.05.010>.
- Hu, J. H., Miller, S. M., Geurts, M. H., Tang, W., Chen, L., Sun, N., et al., (2018). Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature*, 556, 57–63. <https://doi.org/10.1038/nature26155>.

- Huang, X. Z., Zeng, X. F., Li, J. R., & Zhao, D. G., (2017). Construction and analysis of tify1a and tify1b mutants in rice (*Oryza sativa*) based on CRISPR/Cas9 technology. *J. Agric. Biotechnol.*, 25, 1003–1012. 10.3969/j.issn.1674-7968.2017.06.015.
- Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A., (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. *Journal of Bacteriology*, 169, 5429–5433.
- Jia, H., Orbovic, V., Jones, J. B., & Wang, N., (2016). Modification of the PthA4 effector binding elements in type I CsLOB1 promoter using Cas9/sgrRNA to produce transgenic Duncan grapefruit alleviating XccΔpthA4:dCsLOB1.3 infection. *Plant Biotechnol. J.*, 14, 1291–1301.
- Jia, H., Zhang, Y., Orbovic, V., Xu, J., White, F. F., Jones, J. B., & Wang, N., (2017). Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. *Plant Biotechnol. J.*, 15, 817–823.
- Jiang, F., & Doudna, J. A., (2017). CRISPR-Cas9 structures and mechanisms. *Annual Review of Biophysics*, 46, 505–529. <https://doi.org/10.1146/annurev-biophys-062215-010822>.
- Jiang, W., Zhou, H., Bi, H., Fromm, M., Yang, B., & Weeks, D. P., (2013). Demonstration of CRISPR/Cas9/sgrRNA mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic Acids Research*, 41, e188. doi: 10.1093/nar/gkt780.
- Kelliher, T., Starr, D., Su, X., Tang, G., Chen, Z., Carter, J., et al., (2019). One step genome editing of elite crop germplasm during haploid induction. *Nat. Biotechnol.*, 37, 287–292. <https://doi.org/10.1038/s41587-019-0038-x>.
- Knox, A. K., Dhillon, T., Cheng, H., Tondelli, A., Pecchioni, N., & Stockinger, E. J., (2010). CBF gene copy number variation at frost resistance-2 is associated with levels of freezing tolerance in temperate-climate cereals. *Theoretical and Applied Genetics*, 121(1), 21–35. doi: 10.1007/s00122-010-1288-7.
- Koonin, E. V., & Makarova, K. S., (2009). *CRISPR-Cas: An Adaptive Immunity System in Prokaryotes*. F1000 biology reports 1.
- Koonin, E. V., Makarova, K. S., & Zhang, F., (2017). Diversity, classification and evolution of CRISPR-Cas systems. *Curr. Opin. Microbiol.*, 37, 67–78.
- Leenay, R. T., & Beisel, C. L., (2017). Deciphering, communicating, and engineering the CRISPR PAM. *J. Mol. Biol.*, 429(2), 177–191. doi: 10.1016/j.jmb.2016.11.024.
- Li, C. H., Wang, G., Zhao, J. L., Zhang, L. Q., Ai, L. F., Han, Y. F., et al., (2014). The receptor-like kinase SIT1 mediates salt sensitivity by activating MAPK3/6 and regulating ethylene homeostasis in rice. *The Plant Cell*, 26(6), 2538–2553. doi: 10.1105/tpc.114.125187.
- Li, J. F., Norville, J. E., Aach, J., McCormack, M., Zhang, D., Bush, J., Church, G. M., & Sheen, J., (2013). Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nature Biotechnology*, 31, 688–691. doi: 10.1038/nbt.2654.
- Li, J., Meng, X., Zong, Y., Chen, K., Zhang, H., Liu, J., et al., (2016). Gene replacements and insertions in rice by intron targeting using CRISPR-Cas9. *Nat. Plants*, 2, 16139.
- Li, L., Wei, K., Zheng, G., Liu, X., Chen, S., Jiang, W., & Lu, Y., (2018). CRISPR/Cpf1-assisted multiplex genome editing and transcriptional repression in *Streptomyces*. *Appl. Environ. Microbiol.*, 84, e00827–e918. <https://doi.org/10.1128/AEM.00827-18>.
- Li, R., Liu, C., Zhao, R., Wang, L., Chen, L., Yu, W., Zhang, S., et al., (2019). CRISPR/Cas9-mediated SINPR1 mutagenesis reduces tomato plant drought tolerance. *BMC Plant Biol.*, 19, 38. <https://doi.org/10.1186/s12877-018-1627-4>.

- Liu, F., Huang, N., Wang, L., Ling, H., Sun, T., Ahmad, W., et al., (2017). A novel L-ascorbate peroxidase 6 gene, ScAPX6, plays an important role in the regulation of response to biotic and abiotic stresses in sugarcane. *Frontiers in Plant Science*, 8, 2262. doi: 10.3389/fpls.2017.02262.
- Lobell, D. B., & Gourdji, S. M., (2012). The influence of climate change on global crop productivity. *Plant Physiol.*, 160(4), 1686–1697. <https://doi.org/10.1104/pp.112.20829> 8.
- Lou, D., Wang, H., Liang, G., & Yu, D., (2017). OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice. *Front Plant Sci.*, 8, 993.
- Ma, Y., Dai, X., Xu, Y., Luo, W., Zheng, X., Zeng, D., Pan, Y., Lin, X., Liu, H., Zhang, D., et al., (2015). COLD1 confers chilling tolerance in rice. *Cell*, 160(6), 1209–1221. 10.1016/j.cell.2015.01.046.
- Macovei, A., Sevilla, N. R., Cantos, C., Jonson, G. B., Slamet-Loedin, I., Ćermák, T., Voytas, D. F., et al., (2018). Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to rice tungro spherical virus. *Plant Biotechnol. J.*, 16, 918–927.
- Makarova, K. S., Wolf, Y. I., & Koonin, E. V., (2013). The basic building blocks and evolution of CRISPR–Cas systems. *Biochem. Soc. Trans.*, 41, 1392–1400. doi: 10.1042/BST20130038.
- Makarova, K. S., Wolf, Y. I., & Koonin, E. V., (2018). Classification and nomenclature of CRISPR–Cas systems: Where from here? *CRISPR J.*, 1, 325–336. doi: 10.1089/crispr.2018.0033.
- Makarova, K. S., Wolf, Y. I., Iranzo, J., Shmakov, S. A., Alkhnbashi, O. S., Brouns, S. J. J., Charpentier, E., Cheng, D., Haft, D. H., Horvath, P., et al., (2020). Evolutionary classification of CRISPR–Cas systems: A burst of class 2 and derived variants. *Nat. Rev. Microbiol.*, 18, 67–83. <https://doi.org/10.1038/s41579-019-0299-x>.
- Makarova, K., Wolf, Y., Alkhnbashi, O., et al., (2015). An updated evolutionary classification of CRISPR–Cas systems. *Nat. Rev. Microbiol.*, 13, 722–736 <https://doi.org/10.1038/nrmicro3569>.
- Malnoy, M., Viola, R., Jung, M. H., Koo, O. J., Kim, S., Kim, J. S., Velasco, R., & Nagamangala, K. C., (2016). DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. *Front. Plant Sci.*, 7, 1904.
- Maron, L. G., Guimarães, C. T., Kirst, M., Albert, P. S., Birchler, J. A., Bradbury, P. J., Buckler, E. S., Coluccio, A. E., Danilova, T. V., Kudrna, D., Magalhaes, J. V., et al., (2013). Aluminum tolerance in maize is associated with higher MATE1 gene copy number. *Proceedings of the National Academy of Sciences*, 110(13), 5241–5246. doi: 10.1073/pnas.1220766110.
- Marraffini, L. A., & Sontheimer, E. J., (2010). CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. *Nat. Rev. Genet.*, 11, 181–190. <https://doi.org/10.1038/nrg2749>.
- Meng, X., Hu, X., Liu, Q., Song, X., Gao, C., Li, J., et al., (2018). Robust genome editing of CRISPR–Cas9 at NAG PAMs in rice. *Sci. China Life Sci.*, 61, 122–125. <https://doi.org/10.1007/s11427-017-9247-9>.
- Miao, H., Sun, P., Liu, Q., Miao, Y., Liu, J., Xu, B., et al., (2017b). The AGPase family proteins in banana: Genome wide identification, phylogeny, and expression analyses reveal their involvement in the development, ripening, and abiotic/biotic stress responses. *International Journal Molecular Science*, 18(8), 1581. doi: 10.3390/ijms18081581.



- Miao, H., Sun, P., Liu, Q., Miao, Y., Liu, J., Zhang, K., et al., (2017a). Genome-wide analyses of SWEET family proteins reveal involvement in fruit development and abiotic/biotic stress responses in banana. *Scientific Reports*, 7, 3536. doi: 10.1038/s41598-017-03872-w.
- Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., et al., (2018). DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, 32(2), 261–285. doi: 10.1080/13102818.2017.1400401.
- Nandy, S., Pathak, B., Zhao, S., & Srivastava, V., (2019). Heat-shock-inducible CRISPR/Cas9 system generates heritable mutations in rice. *Plant Direct*, 3, e00145. <https://doi.org/10.1002/pld3.145>.
- Navet, N., & Tian, M., (2020). Efficient targeted mutagenesis in allotetraploid sweet basil by CRISPR/Cas9. *Plant Direct*, 4(6), e00233. <https://doi.org/10.1002/pld3.233>.
- Nekrasov, V., Staskawicz, B., Weigel, D., Jones, J. D., & Kamoun, S., (2013). Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nat. Biotechnol.*, 31, 691.
- Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D., & Kamoun, S., (2017). Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Sci. Rep.*, 7, 482.
- Nieves-Cordones, M., Mohamed, S., Tanoi, K., Kobayashi, N. I., Takagi, K., Vernet, A., Guiderdoni, E., et al., (2017). Production of low-Cs<sup>+</sup> rice plants by inactivation of the K<sup>+</sup> transporter OsHAK1 with the CRISPR-Cas system. *Plant Journal*, 92(1), 43–56. doi: 10.1111/tpj.13632.
- Oliva, R., Ji, C., Atienza-Grande, G., Huguet-Tapia, J. C., Perez-Quintero, A., Li, T., Eom, J. S., Li, C., et al., (2019). Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nat. Biotechnol.*, 37(11), 1344–1350. doi: 10.1038/s41587-019-0267-z.
- Ortigosa, A., Gimenez-Ibanez, S., Leonhardt, N., & Solano, R., (2019). Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of SlJAZ2. *Plant Biotechnol. J.*, 17, 665–673.
- Osakabe, Y., & Osakabe, K., (2017). Genome editing to improve abiotic stress responses in plants. In: *Progress in Molecular Biology and Translational Science* (Vol. 149, pp. 99–109). Elsevier: Amsterdam, The Netherlands.
- Ou, W., Mao, X., Huang, C., Tie, W., Yan, Y., Ding, Z., et al., (2018). Genome-wide identification and expression analysis of the KUP family under abiotic stress in cassava (*Manihot esculenta* Crantz). *Frontiers in Physiology*, 9, 17. doi: 10.3389/fphys.2018.00017.
- Pandita, D., (2021). Cas9 technology: An innovative approach to enhance phytoremediation. In: Pirzadah, T. B., Malik, B., & Hakeem, K. R., (eds.), *Plant-Microbe Dynamics: Recent Advances for Sustainable Agriculture*. CRC Press, ISBN1000388913, 9781000388916.
- Pandita, D., (2021). CRISPR/Cas mediated genome editing for improved stress tolerance in plants. In Aftab, T., & Hakeem, K. R., (eds.), *Frontiers in Plant–Soil Interaction: Molecular Insights into Plant Adaptation* (pp. 259–291). Academic Press, Elsevier. <https://doi.org/10.1016/B978-0-323-90943-3.00001-8>.
- Pandita, D., (2021). CRISPR/Cas mediated genome editing technologies in plants. In Aftab, T., & Hakeem, K. R., (eds.), *Plant Abiotic Stress Physiology: Responses and Adaptations* (Vol. 1). Hard ISBN: 9781774630167.
- Peng, A., Chen, S., Lei, T., Xu, L., He, Y., Wu, L., et al., (2017). Engineering canker resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus. *Plant Biotechnol. J.*, 10, 1011–1013.

- Pessina, S., Lenzi, L., Perazzolli, M., Campa, M., Dalla, C. L., Urso, S., Valè, G., et al., (2016). Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. *Hortic. Res.*, 3, 16016.
- Prado, J. R., Segers, G., Voelker, T., Carson, D., Dobert, R., Phillips, J., Cook, K., et al., (2014). Genetically engineered crops: From idea to product. *Annu. Rev. Plant Biol.*, 65, 769, 790. <https://doi.org/10.1146/annurev-ev-arpla-nt-050213-040039>.
- Pyott, D. E., Sheehan, E., & Molnar, A., (2016). Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free *Arabidopsis* plants. *Mol. Plant Pathol.*, 17, 1276–1288.
- Raman, R., (2017). The impact of genetically modified (GM) crops in modern agriculture: A review. *GM Crops & Food*, 8, 195–208.
- Rao, G. J. N., Reddy, J. N., Variar, M., & Mahender, A., (2016). Molecular breeding to improve plant resistance to abiotic stresses. In: Al-Khayri, J., Jain, S., & Johnson, D., (eds.), *Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits*. Springer, Cham. [https://doi.org/10.1007/978-3-319-22518-0\\_8](https://doi.org/10.1007/978-3-319-22518-0_8).
- Rong, Y. S., & Golic, K. G., (2000). Gene targeting by homologous recombination in *Drosophila*. *Science*, 288, 2013–2018.
- Samai, P., Pyenson, N., Jiang, W., Goldberg, G. W., Hatoum-Aslan, A., & Marraffini, L. A., (2015). Co-transcriptional DNA and RNA cleavage during type III CRISPR-Cas immunity. *Cell*, 161, 1164–1174.
- Sedeek, K. E. M., Mahas, A., & Mahfouz, M., (2019). Plant genome engineering for targeted improvement of crop traits. *Front Plant Sci.*, 10, 114. <https://doi.org/10.3389/fpls.2019.00114>.
- Shan, Q., Wang, Y., Li, J., Zhang, Y., Chen, K., Liang, Z., Zhang, K., et al., (2013). Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat. Biotechnol.*, 31, 686.
- Shi, J., Gao, H., Wang, H., Lafitte, H. R., Archibald, R. L., Yang, M., Hakimi, S. M., et al., (2017). ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnology Journal*, 15, 207–216.
- Stella, S., Alcón, P., & Montoya, G. J. N., (2017). Structure of the Cpf1 endonuclease R-loop complex after target DNA cleavage. *Nature*, 546, 559.
- Stockinger, E. J., Skinner, J. S., Gardner, K. G., Francia, E., & Pecchioni, N., (2007). Expression levels of barley Cbf genes at the frost resistance-H2 locus are dependent upon alleles at Fr-H1 and Fr-H2. *The Plant Journal*, 51(2), 308–321. doi: 10.1111/j.1365-313X.2007.0141.x.
- Sun, K., Wolters, A. M., Vossen, J. H., Rouwet, M. E., Loonen, A. E., Jacobsen, E., Visser, R. G., & Bai, Y., (2016). Silencing of six susceptibility genes results in potato late blight resistance. *Transgenic Res.*, 25(5), 731–742. doi: 10.1007/s11248-016-9964-2.
- Sun, Q., Lin, L., Liu, D., Wu, D., Fang, Y., Wu, J., & Wang, Y., (2018). CRISPR/Cas9-mediated multiplex genome editing of the BnWRKY11 and BnWRKY70 genes in *Brassica napus* L. *Int. J. Mol. Sci.*, 19, 2716.
- Sun, Y., Zhang, X., Wu, C., He, Y., Ma, Y., Hou, H., et al., (2016). Engineering herbicide resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate synthase. *Mol. Plant.*, 9, 628–631.
- Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., He, H., Wang, W., Zeng, X., Shao, Y., et al., (2017). Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Scientific Reports*, 7, 14438. doi: 10.1038/s41598-017-14832-9.

- Tang, X., Liu, G., Zhou, J., Ren, Q., You, Q., Tian, L., Xin, X., et al., (2018). A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. *Genome Biol.*, 19, 84.
- Tang, X., Lowder, L. G., Zhang, T., Malzahn, A. A., Zheng, X., Voytas, D. F., Zhong, Z., et al., (2017). A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants. *Nat. Plants*, 3, 17103. <https://doi.org/10.1038/nplants.2017.18>.
- Tashkandi, M., Ali, Z., Aljedaani, F., Shami, A., & Mahfouz, M., (2018). Engineering resistance against tomato yellow leaf curl virus via the CRISPR/Cas9 system in tomato. *Plant Signal. Behav.*, 13, e1525996.
- Tripathi, J. N., Ntui, V. O., Ron, M., Muiruri, S. K., Britt, A., & Tripathi, L., (2019). CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of *Musa* spp. overcomes a major challenge in banana breeding. *Commun Biol.*, 2, 46. doi: 10.1038/s42003-019-0288-7.
- Uga, Y., Okuno, K., & Yano, M., (2011). Drol, a major QTL involved in deep rooting of rice under upland field conditions. *Journal of Experimental Botany*, 62(8), 2485–2494. doi: 10.1093/jxb/erq429.
- Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., et al., (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics*, 45(9), 1097–1102. doi: 10.1038/ng.2725.
- Voytas, D. F., & Gao, C., (2014). Precision genome engineering and agriculture: Opportunities and regulatory challenges. *PLoS Biol.*, 12, e1001877. <https://doi.org/10.1371/journal.pbio.1001877>.
- Vu, T. V., Sivankalyani, V., Kim, E. J., Doan, D. T. H., Tran, M. T., Kim, J., Sung, Y. W., et al., (2020). Highly efficient homology-directed repair using CRISPR/Cpf1-geminiviral replicon in tomato. *Plant Biotechnol. J.*, 1–11. doi: 10.1111/pbi.13373.
- Wang, F. Z., Chen, M. X., Yu, L. J., Xie, L. J., Yuan, L. B., Qi, H., Xiao, M., Guo, W., Chen, Z., Yi, K., et al., (2017). Osarm1, an R2R3 MYB transcription factor, is involved in regulation of the response to arsenic stress in rice. *Frontiers in Plant Science*, 8, 1868. doi: 10.3389/fpls.2017.01868.
- Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., et al., (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. *PLoS One*, 11, e0154027.
- Wang, L., Chen, L., Li, R., Zhao, R., Yang, M., Sheng, J., & Shen, L., (2017a). Reduced drought tolerance by CRISPR/Cas9-mediated SIMAPK3 mutagenesis in tomato plants. *J. Agric. Food Chem.*, 65, 8674–8682. <https://doi.org/10.1021/acs.jafc.7b02745>.
- Wang, L., Chen, L., Li, R., Zhao, R., Yang, M., Sheng, J., & Shen, L., (2017b). Reduced drought tolerance by CRISPR/Cas9-mediated SIMAPK3 mutagenesis in tomato plants. *Journal of Agricultural and Food Chemistry*, 65(39), 8674–8682. doi: 10.1021/acs.jafc.7b02745.
- Wang, X., Tu, M., Wang, D., Liu, J., Li, Y., Li, Z., Wang, Y., & Wang, X., (2018). CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. *Plant Biotechnol. J.*, 16, 844–855.
- Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J. L., (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*, 32, 947–951. doi: 10.1038/nbt.2969.
- Wrighton, K., (2018). Expanding the reach of Cas9. *Nat. Rev. Genet.*, 19, 250, 251. <https://doi.org/10.1038/nrg.2018.15>.

- Wu, W. Y., Lebbink, J. H. G., Kanaar, R., Geijsen, N., & Van, De. O. J., (2018). Genome editing by natural and engineered CRISPR-associated nucleases. *Nat. Chem. Biol.*, 14, 642–651.
- Xu, J., Soni, V., Chopra, M., & Chan, O., (2020). Genetic modification of the HSP90 gene using CRISPR-Cas9 to enhance thermotolerance in *T. Suecica*. *URNCST Journal*, 4(4). <https://doi.org/10.26685/urncst.178>.
- Xu, K., Xu, X., Fukao, T., Canlas, P., Maghirang-Rodriguez, R., Heuer, S., et al., (2006). Sub1A is an ethylene response-factor-like gene that confers submergence tolerance to rice. *Nature*, 442(7103), 705–708. doi: 10.1038/nature04920.
- Ye, Y., Li, P., Xu, T., Zeng, L., Cheng, D., Yang, M., Luo, J., & Lian, X., (2017). Ospt4 contributes to arsenate uptake and transport in rice. *Front in Plant Science*, 8, 2197. doi: 10.3389/fpls.2017.02197.
- Yin, X., Anand, A., Quick, P., & Bandyopadhyay, A., (2019). Editing a stomatal developmental gene in rice with CRISPR/Cpf1. In: Qi, Y., (ed.), *Plant Genome Editing with CRISPR Systems: Methods in Molecular Biology* (Vol. 1917). New York, NY: Humana Press.
- Zafar, K., Khan, M. Z., Amin, I., Mukhtar, Z., Yasmin, S., Arif, M., Ejaz, K., & Mansoor, S., (2020). Precise CRISPR-Cas9 mediated genome editing in super basmati rice for resistance against bacterial blight by targeting the major susceptibility gene. *Front. Plant Sci.*, 11, 575. doi: 10.3389/fpls.2020.00575.
- Zaidi, S., Tashkandi, M., Mansoor, S., & Mahfouz, M., (2016). Engineering plant immunity: Using CRISPR/Cas9 to generate virus resistance. *Front. Plant Sci.*, 7, 1673.
- Zeng, Y., Wen, J., Zhao, W., Wang, Q., & Huang, W., (2020). Rational improvement of rice yield and cold tolerance by editing the three genes OsPIN5b, GS3, and OsMYB30 with the CRISPR-Cas9 system. *Frontiers in Plant Science*, 10. <https://doi.org/10.3389/fpls.2019.01663>.
- Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., Slaymaker, I. M., Makarova, K. S., Essletzbichler, P., Volz, S. E., et al., (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, 163, 759–771. <https://doi.org/10.1016/j.cell.2015.09.038>.
- Zhang, A., Liu, Y., Wang, F., Li, T., Chen, Z., Kong, D., Bi, J., et al., (2019). Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Mol. Breed.*, 39, 47.
- Zhang, T., Zheng, Q., Yi, X., An, H., Zhao, Y., Ma, S., & Zhou, G., (2018). Establishing RNA virus resistance in plants by harnessing CRISPR immune system. *Plant Biotechnol. J.*, 16, 1415–1423.
- Zhang, Y., Bai, Y., Wu, G., Zou, S., Chen, Y., Gao, C., & Tang, D., (2017). Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat. *Plant J.*, 91, 714.
- Zhang, Y., Massel, K., Godwin, I. D., & Gao, C., (2018). Applications and potential of genome editing in crop improvement. *Genome Biol.*, 19, 210. <https://doi.org/10.1186/s13059-018-1586-y>.
- Zhang, Z., Ge, X., Luo, X., Wang, P., Fan, Q., Hu, G., Xiao, J., et al., (2018). Simultaneous editing of two copies of GH14-3-3D confers enhanced transgene-clean plant defense against *Verticillium dahliae* in allotetraploid upland cotton. *Front. Plant Sci.*, 9, 842.
- Zhong, Z., Zhang, Y., You, Q., Tang, X., Ren, Q., Liu, S., et al., (2018). Plant genome editing using FnCpf1 and LbCpf1 nucleases at redefined and altered PAM sites. *Mol. Plant.*, 11, 999–1002. doi: 10.1016/j.molp.2018.03.008.

- Zhou, G., Pereira, J. F., Delhaize, E., Zhou, M., Magalhaes, J. V., & Ryan, P. R., (2014). Enhancing the aluminium tolerance of barley by expressing the citrate transporter genes SbMATE and FRD3. *Journal of Experimental Botany*, 65(9), 2381–2390. <https://doi.org/10.1093/jxb/eru121>.
- Zhou, J., Peng, Z., Long, J., Sosso, D., Liu, B., Eom, J. S., et al., (2015). Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. *Plant J.*, 82, 632–643.

## CHAPTER 12

---

# Epigenetics – The Molecular Tool in Understanding Abiotic Stress Response in Plants

KAKAN BALL and SANJOY SADHUKHAN

*Plant Molecular Biology Laboratory, Department of Botany,  
Raiganj University, Raiganj – 733134, Uttar Dinajpur, West Bengal, India,  
E-mails: ballkakan@gmail.com (K. Ball);  
sanjoysadhukhan@gmail.com (S. Sadhukhan)*

---

### ABSTRACT

Sessile plants are considered the most adaptable organisms of nature, able to change themselves depending on the environmental conditions to which they are exposed. If the conditions are favorable, plants grow effortlessly while in adverse conditions they are unable to maintain their normal activities. ‘Stress’ is regarded as the conditions which are unsuitable for the growth and survival of plants. Plants have to constantly struggle with a plethora of abiotic and biotic challenges of the environment for their survival in nature. On this planet, plants are continuously facing diverse abiotic stresses which impart a detrimental impact on their growth, development, as well as crop production. In response to this stress, plants have developed various mechanisms to survive and adapt to nature. Since plants are sessile, they have a bunch of signaling pathways for perceiving and conveying environmental signals, which results in dynamic reprogramming of transcription. Plant’s development and environmental signals promote epigenetic changes in the genome. Plant cells produce numerous epigenomes due to cellular as well as natural signals; thus, epigenetics becomes the topic of interest. Epigenetics is

a relatively stable and inheritable modification in a gene expression without attributing changes in the DNA sequence. In 1942, Conrad Hal Waddington conceived the word “*epigenetic*.” Epigenetic changes which include Chromatin remodeling (DNA and histone modifications) and RNA-mediated modifications (non-coding RNAs and microRNAs) contribute to gene expression, proliferation of plant as well as growth patterns under stressful conditions. When plants face adverse ecological situations, epigenetic stress-induced gene expression helps them in adaptation. Stress-induced chromatin marks are meiotically transmitted and can transmit the “*memory of stresses*” from parent to offspring plants via transgenerational epigenetic transmission, suggesting the capacity to respond to a plethora of stresses. This information in the role of epigenetics in phenotypic plasticity and heritable variation is vital in understanding how plants can adapt to diverse environmental situations. Keeping these in the background, this chapter intends to depict the recent progress in underlying epigenetic processes implicated in the plant’s reaction against abiotic stress like extreme heat, cold, drought, heavy rainfall, salt, hazardous chemicals, and UV radiation. Further, attempts have been made to illustrate how the epigenetic modifications cross-talks with the biochemical as well as cellular functions of the plants in adapting to different abiotic stresses.

## 12.1 INTRODUCTION

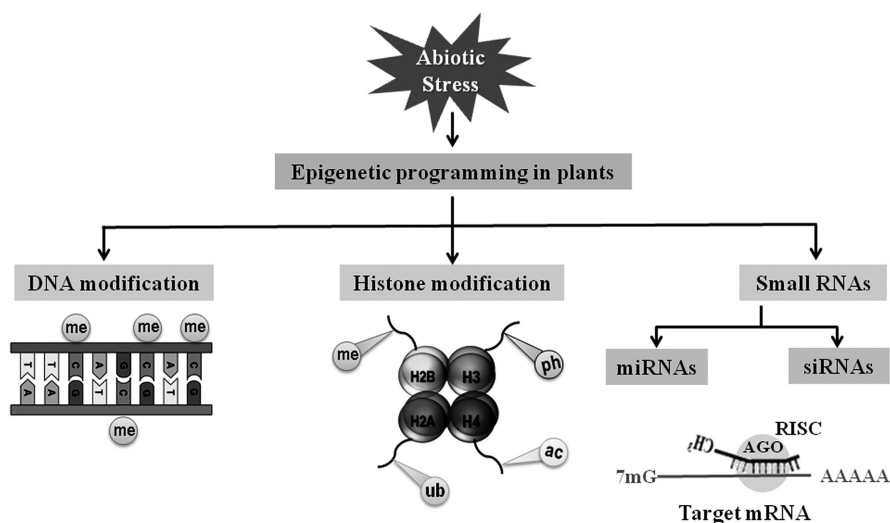
Plants in nature tend to adapt to the diverse environmental cues to which they are exposed. Some of the environmental conditions may be conducive, allowing the plant to grow well, but if conditions are hostile, then the plant will be unable to maintain its growth and normal activities. Stress is defined as an environment that is inappropriate for plant growth and survival. The adverse or critical condition that negatively influences or blocks a plant’s metabolism and growth is known as stress (Lichtenthaler, 1998). There are numerous environmental challenges which include abiotic stress, i.e., salinity, drought, heat, freezing, heavy metals and biotic stresses (Kim, 2021; Kong et al., 2020). During evolution, plants have developed diverse machinery to face different abiotic stresses throughout their life cycle (Kong et al., 2020; Sudan et al., 2018). These mechanisms are regulated by the transcription and translation of stress-induced genes. Since plants are sessile, they are considered as the most adaptable organisms of nature and can sense a change in environmental cues thereby initiates gene expression (Luo et al., 2012), has

a plethora of signaling pathways that eventually leading to transcriptional reprogramming (Bhadouriya et al., 2020). This transcriptional reprogramming brings a global change in the expression of genes by various transcription factors. During this re-programming, the expressions of specific genes are exalted while alternative genes get suppressed in comparison to earlier states. Keeping the genomic sequence unchanged a new molecular characteristic appears which is different from its original form, it also acquires an entirely new molecular sign, which is heritable, this process is known as epigenetic reprogramming (Weinhold, 2006). During the last decades, the basic pathways of stress signaling have been deciphered successively (Zhu, 2016). Several studies have explained the mechanisms of signal transduction involved under plant abiotic stress, of which numerous studies show significant involvement of epigenetic mechanism (Kim et al., 2015; Sahu et al., 2013). The British developmental biologist Conard H. Waddington coined the word “Epigenetics” by joining two words “epigenesis” and “genetics.” Epigenetics is the branch of science that deals with genetically determined characteristics of organisms that are not linked to modifications in DNA nucleotide sequence but can be effectively imprinted within genome (Deans & Maggert, 2015). In other words, epigenetic relates to chemical changes that are both transient and heritable and are not connected with alterations in DNA sequence (Bird, 2007). Over the past decade, the revolution in biological science in the field of molecular epigenetics established it as the “next big thing” (Ebrahim, 2012). The term “Epigenetics” literally denotes “above or on top of genetics” like “events above or outside the gene” (Jablonka & Lamb, 2002). Epigenetic changes occurring on chromosomes include histone modification, DNA methylation, and sRNA-based mechanisms are considered the “three pillars of epigenetics,” they regulate gene expression (Avramova, 2011; Grant-Downton & Dickinson, 2005). The epigenetic control of a plant genome is a complex phenomenon in which a particular group of genes is expressed in response to a specific environmental and developmental situation (Pikaard & Scheid, 2014). Plants employ three types of epigenetic processes to acclimate and sustain in harsh environments: histone modification, DNA methylation, and RNA interference (RNAi) (Singroha & Sharma, 2019). The productivity of crop is hampered due to unfavorable environmental factors. In fact, in the case of numerous major crops there is a reduction in the yield and increase in subsequent price (Lobell & Gourdj, 2012; Lobell et al., 2011; Moore & Lobell, 2015). In response to biotic and abiotic environmental stressors, plants alter their growth pattern as well as physiological processes, to acclimate in the constantly changing



environment. Plants require a continual change at the molecular level and efficiently modify their metabolic pathways (Gallo-Franco et al., 2020). Stress is a situation that can deteriorate plant's fitness to grow and survive that can reduce the yield almost 50% of crop productivity, resulting in scarcity of food (Boyer, 1982). Epigenetic mechanisms regulate the stress-induced genes at the transcriptional and post-transcriptional level (Kim et al., 2015; Luo & He, 2020; Lämke & Bäurle, 2017). Epigenetic regulation enhances the existence of plants by enhancing their forbearance for stresses. Stress signals induce DNA methylation that alter the promoter regions of stress-responsive genes leading to alteration of their expression pattern (Chinnusamy & Zhu, 2009), modification of histone proteins (Gallo-Franco et al., 2020). The enzyme cytosine methyltransferase methylates cytosine residue within the CpG dinucleotide (Bewick et al., 2016; Niederhuth & Schmitz, 2017). DNA covers the histone octamer (H2A, H2B, H3, and H4) while the amino acids of the amino-terminal chain of the globular histone proteins are exposed outside for the post-translational modification (PTMs) and result in alteration of gene expression (Kouzarides, 2007). The expression of genes is suppressed by sumoylation and biotinylation of the amino acids, while acetylation and phosphorylation activate gene expression. DNA replication, transcription, and DNA repair are greatly controlled by histone modification (Nathan et al., 2006; Ransom et al., 2010). DNA methylation is related to the prolonged transgenerational preservation of epigenetic modification and methylation influences the mode of transcription (Gallo-Franco et al., 2020). DNA methylation is the most frequent stress-responsive epigenetic modification, which occurs across plant species and strengthens the significance of this process (Feng et al., 2010). Most of the epigenetic modifications are inherited and perform a pivotal part in plant adaptability (Robertson & Wolf, 2012). Methylation in genomic DNA is transmitted over generations without having any modification in the acquired methylation pattern, this leads to the formation of "epialleles" (Kalisz & Purugganan, 2004). Epialleles may exist above stress-associated genes but maybe even exist in genomic areas which have no relation with the stress response (Verhoeven et al., 2010). Gene expression may be suppressed by transposons, this may be caused by the methylated state of transposons that are located near the genes and even regulate the expression of genes through methylation spread mechanism (Saze & Kakutani, 2007). sRNAs of diverse types, as well as lncRNAs, play an important role in epigenetic processes. Small RNAs change the chromatin conformation and regulates gene silencing through Argonaute mediated complexes along with complementary emerging RNA platform resulting

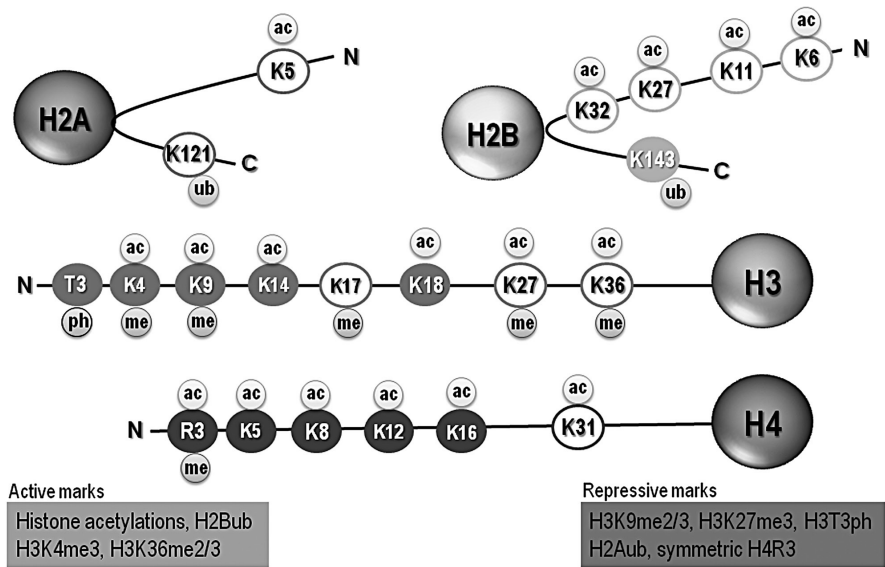
in the engagement of histone as well as DNA methyltransferases (Holoach & Moazed, 2015). These RNAs are produced from double-stranded RNA (dsRNA) precursors as a 20–30 nucleotide long single-stranded RNA. The architecture of chromatin got changed by the histone modification pathway and alters gene expression (Singroha & Sharma, 2019). Gene activation or silencing is associated with the open chromatin conformation or close chromatin structure under the control of different developmental or environmental factors (Figure 12.1).



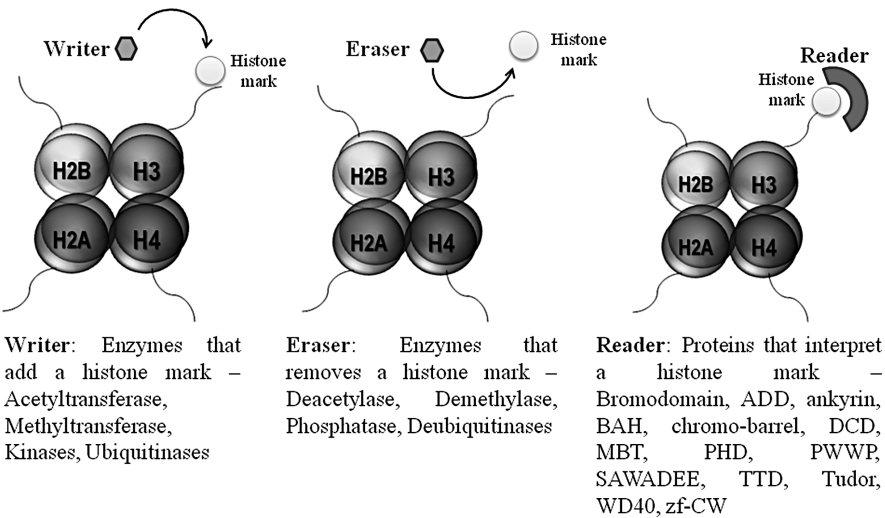
**FIGURE 12.1** Epigenetic programming under abiotic stresses in plants is shown, at three levels: DNA modifications, histone modifications, and small RNAs.

*Abbreviations:* 7mG: 7-methylguanosine cap; AGO: argonaut; H2A: histone 2A; H2B: histone 2B; H3: histone 3; H4: histone 4; ac: acetyl; me: methyl; ph: phosphate; ub: ubiquitin; miRNAs: microRNAs; RISC: RNA-induced silencing complex; siRNAs: small interfering RNAs.

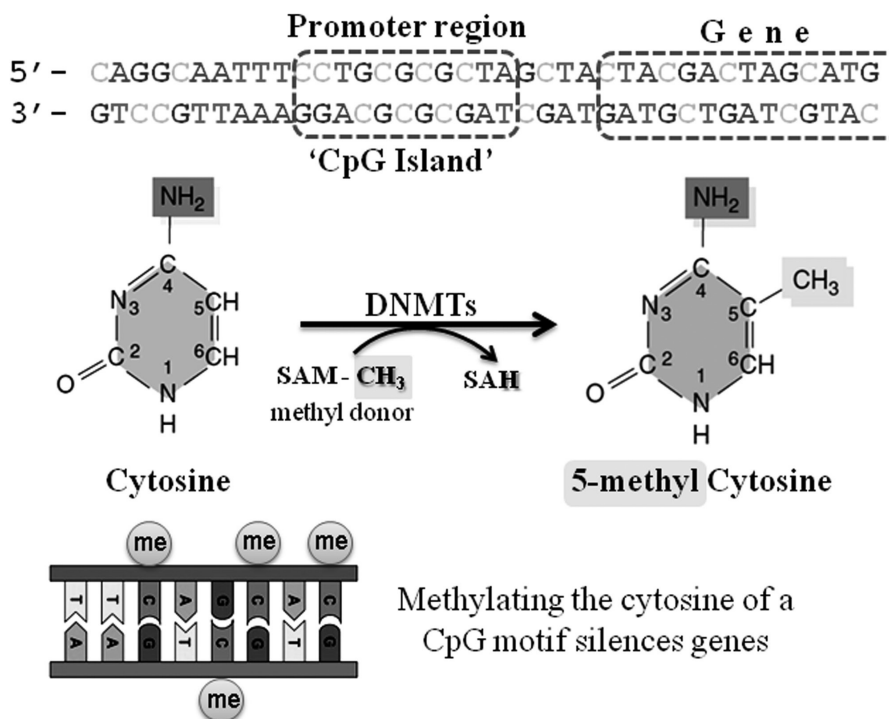
Recently few studies have been conducted for the description of the epigenomic control stress in plants that helps to understand how chromatin modification takes place by diverse abiotic stresses, by their corresponding transcriptional and physiological involvement (Demirel et al., 2020; Zhao et al., 2021) (Figures 12.2–12.4).



**FIGURE 12.2** Modification of amino acid sites of the core histone involved in abiotic stress response are shown in white letters (R: arginine; K: lysine).



**FIGURE 12.3** Epigenetic regulators involved in abiotic stress response. The combined activity of erasers, readers, and writers regulate the level and type of histone modifications.



**FIGURE 12.4** DNA methylation: its mechanism and site.

## 12.2 DIFFERENT ABIOTIC STRESSORS

Plants face a gamut of abiotic stresses in the environment, i.e., salinity, drought, submergence/flooding, extreme temperature (heat and freezing), UV radiation, heavy metals, etc. Some of the stresses are natural while others are due to anthropogenic activities. These stress conditions negatively influence or blocks the plant's metabolism and growth (Lichtenthaler, 1998). The environmental stresses are categorized as biotic and abiotic stress, along with the two the abiotic stress causes major crop loss (Gull et al., 2019). Major numbers of abiotic stresses are compiled and summarized in subsections.

### 12.2.1 SALINE STRESS

In many parts of the world, the plant faces major abiotic stress which is due to the presence of too much amount of salt content in the soil, known

as saline stress. This ultimately results in the inhibition of plant growth and leads to death, thereby resulting in a reduced yield of crop plants. Plants may be categorized into two major classes: (i) halophytes; and (ii) glycophytes. Halophytes are the plants that can tolerate salinity and survive on the other hand, glycophytes are the group of plants that cannot withstand saline stress, and as a result, they die eventually. Glycophytic plants account for a large portion of agricultural plants (Gupta & Huang, 2014). Thus, salinity is among the most deleterious environmental stressors that hinder agricultural yield across the world (Flowers, 2004; Munns & Tester, 2008). Salinity stress contributes to the alteration of different metabolic as well as cellular activities, the intensity and period of the stress, hamper crop production (James et al., 2011; Rozema & Flowers, 2008). Abnormal intake of sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ) ions causing cytotoxicity and creates an imbalance of nutrition, which results in a decrease in plant growth as well as development through water stress. Salinity goes hand in hand with oxidative stress since reactive oxygen species (ROS) are formed along with increased salinity (Isayenkov & Maathuis, 2019). The effect of salinity in plants has been divided into two phases of plant growth. Stomatal openings close and the leaf expansion is repressed in phase I, which takes place in a short time, i.e., minutes or few days. While in phase II, cytotoxic ions build up especially in mature foliages producing premature senescence, which downtrend metabolic processes, decrease production eventually kills the plant (Kotagiri & Kolluru, 2017). The growth and development in agricultural plants get hindered due to toxicity caused by ions (especially  $\text{Na}^+$  ions), hyper osmotic stress and oxidative damage in a highly saline environment. Cellular  $\text{Na}^+$  ion homeostasis is maintained by the secondary messenger  $\text{Ca}^{2+}$  along with Salt Overly Sensitive proteins.  $\text{Ca}^{2+}$  signals are decoded by  $\text{Ca}^{2+}$  sensors. The signal is interpreted into physiological, metabolic, and molecular adaptations by calcineurin B-like protein 10 (CBL10) (Zhu, 2016).  $\text{Na}^+$  influx is mediated by high-affinity  $\text{K}^+$  transporters 1 (HKT1). In *Arabidopsis*, considerable methylation is shown at 2.6 kb upstream of the start site of small RNA which represses the expression of *AtHKT1* in shoots (Baek et al., 2011). The expression of HKT1 is increased due to a decrease of DNA methylation level in RdDM mutant *rdm2*; indicating *AtHKT1* gene expression is negatively regulated by RdDM, this control process is even shown for wheat (Kumar et al., 2017). In the roots and shoots of salt-tolerant and salt-sensitive plants, the expression of *TaHKT2;1* and *TaHKT2;3* gets repressed. This repression is due to cytosine methylation as a result of salt stress, although the modulation of DNA methylation is not the reason for

root-specific repression of *TaHKT1* (Kumar et al., 2017). In saline conditions, plants undergo histone modification in addition to DNA methylation (Kim et al., 2015). To acclimate in the constantly changing environment, acetylation and deacetylation takes place by HATs and HDACs correspondingly. Interestingly, high salinity is responsible for the decline of repressive marks H3K9me2 and H3K27me3 deposition on stress-responsive genes while it influences the accumulation of dynamic histone inscription H3K9K14Ac and H3K4me3 on that salt stress-responsive gene (Chen & Wu, 2010; Sokol et al., 2007; Yolcu et al., 2016). At the elevated amount of NaCl and ABA represses HD2 family deacetylase, i.e., *HD2C* it is reported that the *hd2c* mutant is also sensitive to NaCl (Ming Luo et al., 2012). The expression of the *Arabidopsis HKT<sub>1</sub>* gene is activated by the addition of salt when histone H3 lysine tri-methylation (H3K27me3) is removed from the *HKT<sub>1</sub>* gene body (Sani et al., 2013). Plants under salinity stress result in another type of histone modification, i.e., H3Ser-10 phosphorylation that is associated with chromatin compactness. It was analyzed that H3Ser-10 phosphorylation, H3 and H4 acetylation show an over-expression under different abiotic stress (Sokol et al., 2007). The epigenetic alterations that are mentioned do not act in the same manner as its regulatory mechanism varies depending upon diverse abiotic stress (Kim et al., 2015; Sokol et al., 2007).

#### 12.2.1.1 DROUGHT

Drought is a condition when a region faces inadequate precipitation. It has an adverse effect on the ecosystem and agriculture and the economy of the region. Drought or water deficit is among the major abiotic stresses for the plant's growth, development. The warm-dry areas of the world greatly face drought stress (Pourdad & Beg, 2003). The productivity of crops is adversely hampered because such stresses disrupt the normal synchronized physiological equilibrium (Gaspar et al., 2002). Drought stress takes place due to the unavailability of water in the soil on the other hand increasing the temperature of the environment causes continuous water loss through transpiration and/or evaporation. Drought stress is experienced by most plant species; however, the amount of it varies between species to species and even in the same species (Jaleel et al., 2007). Drought stress causes the decrease in water level in the plant cell, drop off water potential and loss in turgor, decrease in cell elongation and growth, stomatal closure. Dreadful water stress disturbs photosynthesis, metabolism as a result necrosis takes

place (Jaleel et al., 2008). The consequences of imminent climate change, drought will have an intense impact on crop productivity in the future (Shanker et al., 2014). Drought stress intensity and drought stress-responsive genes have a complimentary connection (Kim et al., 2012). Some drought stress-responsive genes that encode functional and regulatory transcription factors in response to drought have been studied (Shinozaki & Yamaguchi-Shinozaki, 2007). Histone modification and chromosome density occur as a response to drought stress (Kim et al., 2012). Under severe drought stress the upregulated genes, such as *RD20* and *RD29A* shows histone modifications H3K4me3 and H3K9ac compared to the average drought situation (Kim, 2021). Additionally, remarkable nucleosome loss occurred in the *RD29A* region of the gene, while a small loss of nucleosome from the same region occurs under moderate drought conditions. These results reveal that the intensity of drought stress affects epigenetic responsiveness. Nucleosomal substitution and strong histone deacetylation are necessary to complete repression of stress-upregulated genes and to reset the chromatin structure under non-drought situation (Kim et al., 2012). Plants can regulate the repeated cycles of stress by modifying the expression pattern of stress-induced genes. The expression of “memory genes” takes place at extremely increased or decreased through consequent dehydration, thus allowing plants to respond quickly during future drought stress. This phenomenon is known as “*stress memory*” which has several synonyms, i.e., imprinting, priming, training, and acclimation. In mammals, the gained stress memory can be rearranged upon healing, consequent development, and meiotic cell division. How plants control the resetting process during meiosis and transmitted to the next generation remains a question to be answered yet (Kim et al., 2020).

### **12.2.2 EXTREME TEMPERATURE STRESS**

Heat stress and cold stress are terms used to describe when temperatures increase or fall dramatically beyond or below a threshold level above a prolonged time, causing serious harm to the plant's growth and development. Generally, a sudden increase, i.e., above 10–15°C above the ambient temperature disturbs the homeostasis of the plant, which is regarded as heat shock or heat stress. This heat stress act as an ultimatum to crop production globally. In the last couple of years, several research on heat and cold stress response mechanisms are demonstrated along with their epigenetic regulation (Ding et al., 2019; Driedonks et al., 2015; Guo et al., 2018; Liu et al., 2018).

### 12.2.2.1 COLD STRESS

Plant growth and development is adversely impacted with cold climatic condition (Chew & Halliday, 2011). Plants have developed an advanced mechanism to sense the alteration in temperature and adjust their physiology accordingly for survival (Chinnusamy et al., 2006; Kaplan et al., 2004). Plants in the temperate regions use the process of cold acclimation to survive under freezing stress. The expression of cold-responsive (COR) genes is turned on. The expression of CORs is controlled by C-repeat-binding factors (CBFs) (Liu et al., 2019). Like *Arabidopsis* sp. homologous CBF is also found in different plant species, e.g., *Brassica campestris* (Wang et al., 2014), *Hordeum vulgare* (Marozsán-Tóth et al., 2015), Rapeseed (Jaglo et al., 2001), tomato (Zhang et al., 2004), rice (Dubouzet et al., 2003). Cold stress induces the transcription factor CBF binds with the promoter region of COR genes containing C-repeat (CRT)/DREs. When *Arabidopsis* is exposed to cold temperatures, the repressive histone mark, i.e., H3K27me<sub>3</sub> on the COR genes (*COR15A* and *ATGOLS3*) diminish slowly (Kwon et al., 2009). In *Arabidopsis* PICKLE (PKL) is a well-reported, ATP dependent, CHD3-type chromatin remodeling factor that regulates growth and development also RNA-directed DNA methylation (RdDM). PKL has a significant part in cold stress response. It regulates numerous COR genes, as well as *RD29A*, *COR15A*, and *COR15B*, by regulating the expression of the *CBF3* gene (Yang et al., 2019). Under cold stress there is a decrease in the deposition of histone repressive mark H3K27me<sub>3</sub> on the COR genes *COR15A* and galactinol synthase 3 (*GOLS3*), this mark is irreversible even under normal temperature, indicate H3K27me<sub>3</sub> can function as a memory indicator (Kwon et al., 2009). Moreover, histone methylation, histone acetylation even plays a crucial role in low-temperature stress response on a significant percentage of cold-induced genes (Park et al., 2018). On the promoter region of different COR genes like *COR15A* and *COR47* histone acetylation takes place under low-temperature treatment (Pavangadkar et al., 2010). Histone acetyltransferase (HATs) and histone deacetylases (HDACs) regulate the histone acetylation dynamically. Cell membranes are frequently damaged under cold stress. Malondialdehyde (MDA) is a key indicator of membrane system damage and degradation in cellular metabolism. MDA levels remain substantially greater under low-temperature stress when compared to the control level. Cold stress treatment led to a gradual increase in malondialdehyde (MDA) concentration when *Arabidopsis* histone deacetylase 2D (HD2D) was overexpressed (Chang et al., 2020).



The role of miRNA-based gene control under low-temperature stress has been proven in numerous research works. Different functional investigations have sought to uncover miRNA-target interactions as well as the relevance of numerous miRNAs in cold-stress modulation. Sunkar & Zhu (2004) were the first to show that miRNAs have a role in the modulation of cold stress. In rice 18 COR microRNAs have been identified under low-temperature stress at 4°C such miRNAs act as predominant regulators of plant responses to low-temperature stress (Lv et al., 2010). At the onset of the productive phase in the rice plant, there is a sharp expression of the COR miR812q is comprised of unique sequences and is derived from the sequence-diverged region. Cold stress significantly elevates the expression of miR812q, which down-regulates *CIPK10*. The Ser/Thr protein kinase domain of CIPK (calci-neurin B-like (CBL) protein-interacting protein kinase) proteins is activated by interacting with CBL, which includes four EF-hands for Ca<sup>2+</sup> binding. Under cold stress, miR812q may be required to alter CBL-CIPK signaling (Jeong & Green, 2013). In tea (*Camellia sinensis*), under cold stress 31 miRNAs are upregulated and while 43 miRNAs get downregulated. Similarly, increased expression was shown by 192 miRNAs and 205 miRNAs revealed a decreased expression in wild tomato (*Solanum habrochaites*) (Cao et al., 2014).

#### 12.2.2.2 HEAT STRESS

Increased occurrences of heatwaves and rising global temperatures influence crop productivity and world food production severely. As global warming progresses, heat stress (HS) seems to be a more serious food security issue. Therefore, heat stress response and its transcriptional network have been studied thoroughly (Ohama et al., 2017). The principal transcriptional regulators in heat stress response (HSR) are heat shock transcription factor A1s (HsfA1s). The complexity of heat stress response is decided by the multilevel regulation of HsfA1s. HsfA1s are partially regulated by phosphorylation or dephosphorylation, SUMOylation, and protein-protein interaction. Heat Stress Response genes get activated by some heat-induced TFs, e.g., dehydration responsive element binding protein 2A (DREB2A), multiprotein bridging factor 1C (MBF1c), HsfA2, Hsf7s, and HsfB<sub>s</sub>. It is predicted that the expression of such heat-induced TFs is directly regulated by HsfA1s (Ohama et al., 2017). Histone dynamics and the RdDM route are included in HSR (Lämke et al., 2016; Olga et al., 2013; Yang et al., 2018). Heat shock proteins

(HSPs) have a critical function in heat sufferance. Generally, the main role of HSPs is controlling protein winding and unwinding (Singh et al., 2016). Heat stress generates a continued aggregation of H3K9ac and H3K4me3 on HSP18, HsP22.0, APX2 and HSP70 genes within these genes (Lämke et al., 2016). The *Arabidopsis* imprinted *SUPPRESSOR OF DRM1 DRM2 CMT3* (*SDC*) gene has been stimulated by prolonged heat stress, which encodes a suspect F-Box protein and is inhibited through DNA methylation during vegetative growth (Sanchez & Paszkowski, 2014). The function of these genes is to function a significant part to overcome heat stress (Olga et al., 2013).

To overcome heat stress, ncRNAs especially miRNAs, play an important part. A large number of heat-induced miRNAs is identified in plants (Cao et al., 2014). Some of them target a diverse range of transcription factors homeobox leucine-zipper proteins, F box proteins and protein kinases such heat stress-responsive miRNAs are miRNA156, miR166, and miR393 families. *Arabidopsis* expressing a miR398-resistant version of CSD (copper/zinc superoxide dismutase) are further susceptible to heat stress. But to regulate MYB-like family transcription factors miR159 is repressed in response to heat stress (Ravichandran et al., 2019).

### 12.2.3 UV RADIATION STRESS

The depletion of the stratospheric ozone layer causes a major hazard to plants as well as animals due to UV radiation from the sunlight with a wavelength (10–400 nm). The UV component of sunlight causes a variety of damage that can disrupt cellular and structural integrity, as well as growth and development. Though many plants are unaffected by a slight increase in exposure to UV radiation as it may depend on the area, species, and cultivator. But the composition of the ecosystem is constantly changing which may affect the important crops (Bornman, 1991). UV-B stress causes a change in chromatin structure and histone H3 acetylation not DNA methylation, which results in rapid and heritable changes of epigenetic control in a silent reporter gene (Lang-Mladek et al., 2010). The epigenetic changes due to UV radiation stress are linked to transcriptional activation, repression, or both and chromatin remodeling. Numerous genes are deregulated by UV-B (280–310 nm) and UV-C (<280 nm) exposure. DNA methylation, or 5-methylcytosine (5mC), is revealed to be an important epigenetic marker under UV stress. DNA methylation profiles are due to change in methylation and demethylation processes,

both of which are necessary due to stable inhibition in transposable element (TE) silencing and gene activity modulation. *Glechoma longituba* (Nakai) Kuprian clonal plants can remember prior UV-B stress. The parental ramets were exposed to UV-B stress once or many times (20 and 40 repetitions). It was observed that UV-B stress reduced the degree of DNA methylation in parental ramets, and the reduction was boosted by increasing the number of UV-B treatments. Recurrently stressed parents' epigenetic variation lasted a prolonged period, whereas singly stressed parents' epigenetic heterogeneity was just temporary. The research result indicated that the growth of progeny and genets was affected by parents' exposure to UV stress. This effect could be influenced by parental epigenetic imprinting and the transgenerational effect (Zhang et al., 2021).

A vast variety of microRNAs have been found in response to UV-B exposure, including miR156, miR159, miR164, miR167a, miR171, and miR395. When plants are exposed to UV-B radiation at various intervals of time, miR159, miR167a, and miR171 are elevated, while miR156, miR164, and miR395 are repressed. In a study, when wheat was exposed to UV-B radiation a new wheat miRNA, Tae-miR6000 was identified, including their diverse expression was confirmed (Wang et al., 2013).

#### **12.2.4 HEAVY METAL (HM) STRESS**

Natural elements are known as “heavy metals” have a relatively large atomic mass as well as a high density. HM stress developed as a serious issue in several terrestrial ecosystems around the world. Due to vast industrialization and HM accumulation in soil has negative consequences on soil and crop yield (Shahid et al., 2015). Several natural factors such as volcanic eruption, weathering of rocks is the reason for HM accumulation in the soil along with these anthropogenic activities like mining, chemicals used in the agricultural field and industrial pollution are the major factor of heavy metal concentration in soil. Generally, HMs has a molecular weight over 20 g/mol and/or density of at least 5 g/cm<sup>3</sup> to distinguish them from, “light” metals. An increase in heavy metal's concentration in soil results in genotoxicity, cytotoxicity, and mutagenic effect on both plants and animals. Based on physiological conditions heavy metals are classified into two classes: (i) essential elements; and (ii) non-essential elements. Although essential elements are required in a small quantity in plants, a high concentration of both types of results in toxicity and act as a barrier to plant growth and

development (Rascio & Navari-Izzo, 2011). HMs in acidic soil is solubilized and the phytotoxic cation species, e.g., magnesium ( $Mg^{2+}$ ), calcium ( $Ca^{2+}$ ), phosphorus (P), sodium ( $Na^+$ ) as well as aluminum ( $Al^{3+}$ ) imparts a negative effect on plant's physiology and leading to a loss in crop yield (Fryzova et al., 2017; Samac & Tesfaye, 2003). Approximately 30% of the fertile land of the world is acidic soil, which is classified as ultisol or oxisol with pH lower than 5.5 and 13% of staple food crops are obtained from this land (Bojórquez-Quintal et al., 2017; Rahman et al., 2018). The toxic effect of aluminum (Al) in the soil is a major bottleneck in agricultural productivity (Kochian et al., 2015; Zheng, 2010).

The importance of epigenetic mechanisms in plant stress response is being highlighted by an increasing number of research studies. Plants' responses to HM stressors are influenced by epigenetic mechanisms. The major epigenetic approaches include: (i) plants are guarded against probable DNA damage induced by metal ions by spontaneous DNA methylation around the genome using epigenetic inscriptions; as well as (ii) transposon and stress-responsive genes are regulated by epigenetic alterations (Gallo-Franco et al., 2020). HMs stimulates DNA methylation alterations, which are primarily related to hypomethylation. Heavy metal stress affects DNA methylation of metal-tolerant Hemp (*Cannabis sativa* L.) and metal-sensitive Clover (*Trifolium repens* L.) plants. When plants maintained in soils polluted with various amounts of  $Ni^{2+}$ ,  $Cd^{2+}$ , and  $Cr^{6+}$  revealed different levels of 5-methylcytosine (5mC) in the root DNA. For the same quantity of root DNA, the methylation level in hemp is roughly three times greater than in clover. Heavy metal administrations resulted in a substantially dose-related drop in 5mC in both hemp and clover ranging from 20 to 40%. Dose-dependent hypermethylation occurs in both clover and hemp due to heavy metal treatment which suggests that natural methylation level is the main reason for metal stress sufferance in hemp (Aina et al., 2004). When the nuclei extracted from foliage cells of *Noccaea caerulescens* plants (a Ni hyperaccumulator species) cultivated in a habitat with high Ni were found to be intact in comet assay at basic pH, but the nuclei of non-tolerant *Arabidopsis thaliana* subjected to Ni were significantly degraded. This was due to considerable hypermethylated DNA in *Noccaea caerulescens* plants compared to *Arabidopsis thaliana* at the genome level. Furthermore, the qRT-PCR results demonstrated that in the case of excess amount of Ni in foliages, the genes MET1, DRM2, and HDA8 of *N. caerulescens* are involved in the up-regulation of epigenetic DNA and histone modification. These epigenetic alterations could be a defense mechanism that allows plants to survive in harsh environments by

avoiding genome destabilization as well as Ni ion mediated disruption of DNA structure (Gulli et al., 2018). Pine trees (*Pinus silvestris*) were found to withstand high ionizing radiation, have much more hypermethylated loci than less adapted plants (Kovalchuk et al., 2003; Volkova et al., 2018).

Plants exposed to HM have miRNA-directed transcriptional and post-transcriptional regulation of gene expression through base-pairing with its focused mRNAs (Min Yang & Chen, 2013). There is an involvement of mi-RNA in response to Cd which is evidenced by abnormal miRNA in stressed plant cells and tissue. It is documented when rapeseed (*Brassica napus*) is treated with Cd exposure there is an up-regulation of the bna-miR393 gene in foliages, bna-miR156a, bna-miR167a/c in roots and foliages, bna-miR164b and bna-miR394a/b/c across tissues whereas down-regulation of miR160 is noticed by Huang et al. (2010). Mercury (Hg) is an extremely poisonous element, with its ionic type ( $\text{Hg}^{2+}$ ) is considerably common in soil. It is also the most accessible form for plants, as it is rapidly absorbed by the plant's root and apical parts. Various toxic responses such as growth inhibition, an anomalous function of vascular bundle, deformity of the cell, reduction in chlorophyll amount as well as accumulation of ROS due to high Hg concentration in the soil. In response to high exposure to Hg stress, different plant species reveals differential miRNAs expression. The barrel-clover plants (*Medicago truncatula*) when exposed to high Hg stress, conserved miRNAs along with non-conserved miRNAs expressed differentially. The vast majority of the new candidate miRNAs were shown to be significantly controlled by the heavy metal mercury Hg (II), including 12 miRNAs showing especially sensitive to Hg treatment (Zhou et al., 2012). Only next to iron, Mn is the abundant transition element present on the planet which is widespread in soils, sediment, and water as well as biological components. Mn is an important co-factor for many enzymes, like the mitochondrial superoxide dismutase. Excess Mn in plants causes toxicity, on the other hand, has a variety of morphological consequences, including chlorosis and necrosis, crinkled leaves, and brown patches, and, eventually, growth suppression. Valdés-López et al. in 2010 investigated that the differentially expressed pattern of miRNAs in common bean (*Phaseolus vulgaris*) in response to Mn stress and found 37 miRNAs. Around 11 miRNAs were active under Mn stress, whereas another 11 were found to be suppressed. Mn-responsive miR1508, miR1515, miR1510/2110, and miR1532 have been discovered, including calcium-dependent protein kinase, heat shock proteins, nucleotide-binding region leucine-rich repeat resistance-like proteins, and receptor kinase protein as candidates, correspondingly (Valdés-López et

al., 2010). Arsenic (As) is a non-essential, extremely toxic, class-I carcinogenic metalloid that is harmful to all living things, including plants. It is that is widely dispersed in the earth's crust and poses serious health risks to humans when it enters the food chain via contaminated food crops. Arsenic reaches plants through phosphate transporters or aquaglycoporins as a phosphate analog. Arsenic uptake in plant tissues has a negative impact on plant metabolism and causes a variety of physiological as well as structural problems (Srivastava et al., 2012). For exploring more about miRNA's function in selenium's antagonistic impact on arsenic stress of rice saplings, researchers discovered that combination of Se and As reduced detrimental roles of As on sapling sprouting, root-shoot development, chlorophyll, and protein content. When rice seedlings were exposed to As, Se, or As with Se, miRNA microarray analysis revealed significant modulation of 46 miRNAs relative to the unmodified control. Depending on the dosage, 18 of such miRNAs had varying levels of regulation. There may be a probable effect of miR395 and miR398 in the counter effect on the unfavorable response of As in rice seedlings in the existence of Se (Pandey et al., 2015). The impact of As-stress on rice plants was investigated at several developmental phases (saplings, tillering, and blooming) and varied tissues of the foliage, stem, and root under a 25 M arsenite [As(III)] exposure (at 0, 24, 48, and 72 h). Expression of Osa-miR156j, an associate of the miR156 gene group that regulates plant growth and development was studied under the effect of arsenic toxicity at various phases of physiological growths. In various developmental phases, the expression of Osa-miR156j revealed a temporal ↓ in multiple organs. In comparison to other tissues, the ↓ was evident high in root tissues at saplings, plant with a tiller, and blooming phases in 0–72 h with arsenite treatment. From this study, it is evident that the microRNA Osa-miR156j could be beneficial in the generation of arsenic-tolerant plants (Pandey et al., 2020). Aluminum (Al) is a major poisonous factor for crop production since they adhere to –COOH and PO<sub>4</sub> groups in the root cell wall, causing architectural alterations, inhibition of cell wall extension, and reduced root expansion. Through miRNA expression patterns and *in silico* study of target genes, a group of researchers studied the probable Al<sup>3+</sup> stress tolerating mechanisms of sugarcane. They found miR395 is associated with Al<sup>3+</sup> elimination, while miR160, miR6225-5p, and miR167 were found to be involved in lateral root development, conferring genotype tolerance. These results could aid funding to future genetic improvement programs focused on developing sugarcane genotypes that are resistant to abiotic stress, as well as biotechnological efforts aiming at miRNA suppression or gene upregulation.

In a recent research study on the plant *Brassica juncea*, 11 recognized and unique miRNAs were found under Cd stress-responsive miRNAs and transcripts. Among this bra-miR172b-3p target for ATCCS (copper chaperone for superoxide dismutase) and bra-miR398-3p target for FLA9 (Fasciclin-Like Arabinogalactan-protein 9) were two of the target miRNAs that were ↓ in Cd-exposed foliages the putative Cd-specific resistance inhibitor bra-miR172b-3p was identified to be adversely controlled in ATCCS in response to Cd stress. These results will aid in a deeper comprehension of the Cd-induced miRNA modulatory circuits of mustard (Liu et al., 2021).

### 12.2.5 SUBMERGENCE/FLOODING STRESS

The impacts of global warming have led to severe drought in several parts of the planet, whereas the unforeseen occurrence of floods has been a catastrophe in different corners of the globe. As per the Food and Agriculture Organization (FAO) of the United Nations, floods caused roughly US \$19 billion in damages in developing world agriculture from 2005 to 2015. Depending on the flooding scenario, adverse impacts may vary, affecting mainly the roots (waterlogging) else whole plant (part or total plant drown) (Sasidharan et al., 2017). Submergence generates a large number of challenges, including nutritional deprivation, hypoxia, infection, and low light, all of that impede plant's enlargement, differentiation including productivity. Gaseous exchange in the soil is subsequently hampered under waterlogging conditions. As a result, there is a large loss of oxygen (O<sub>2</sub>) and accumulation of carbon dioxide (CO<sub>2</sub>) due to microorganisms and root respiration (Bailey-Serres & Voesenek, 2008). Flooding enhances the development of many pathogens which causes plant disease (Gravot et al., 2016). This deleterious effect may be prevented by Hypoxia-triggered gene expression by which many proteins are synthesized and that enhance the plant immunity (Hsu et al., 2013). Hypoxia is characterized by a reduction in the amount of oxygen surrounding a plant's roots; its complete absence (anoxia) causes significant ecological stress. Hypoxia prompts the roots to switch between oxygen-requiring to non-oxygen requiring metabolic-process, resulting in substantial limits on ATP synthesis (Barrett-Lennard, 2003; Teakle et al., 2006). Submergence-responsive genes such as ADH1 and PDC1 have enhanced expression in rice. Expression of the *Oryza sativa* ADH1 and PDC1 genes was enhanced under submergence conditions, as evidenced by higher recruitment of RNA polII on the gene loci. The H3K4 residue in the 5'- and

3'-CDS of ADH1 as well as PDC1 were methylated between H3K4me2 to H3K4me3, whereas acetylation of H3 increased with longer exposure across the ADH1 and PDC1 genes. Environmental stressors govern progressive and bidirectional changes in H3K4 methylation and H3 acetylation under stress-responsive genes of vascular plants (Tsuji et al., 2006). Plant cells must recognize changes in O<sub>2</sub> levels to activate hypoxia-reoxygenation reactions. For plant cells to sense O<sub>2</sub> levels, the integrity of transcriptional regulators of group VII of the ethylene response factor family (*ERFVIIIS*) is crucial. Ethylene response factors VII (*ERFVIIIs*) play a key role in ethylene signaling and plant flooding responses; have an AP2 DNA-binding domain. In *Arabidopsis thaliana* based on stage of development plants are confronted by stress conditions. ERF-VII proteins have a dual role under submergence: as activator of the hypoxic response and as negative modulator of the oxidative-stress associated gene (Giuntoli et al., 2017).

Azahar et al. in 2020 discovered and characterized submergence sensitive miRNAs in maize seedlings using two sRNA libraries of normal and treated (submergence) maize roots. They discovered 524 identified miRNAs as well as 48 new ones. Submergence responsive miRNAs have been linked to a number of cellular activities, involving growth and development (miR172a, miR393b-3p), hormone signaling routes (miR159c,f, miR160f-5p, miR167d-3p, miR172a, miR393a-5p), and oxidoreduction equilibrium (miR164a,b,c-5p, miR166h, a-3p, miR167 (miR156d-3p, miR159c,f, miR169c-3p Overexpression of ERTF RAP2-7 ISOFORM X2 by miR172a may have been a basic requirement for the change from oxygenated to less efficient non-oxygenated fermentation, resulting in limited plant development during submerged conditions. The data suggest that dysregulation of the ROS scavenging system may be a key determinant in maize seedlings' poor performance under submerged conditions. The study contributes new perspectives of maize saplings' post-transcriptional stress response mechanisms to submergence stress.

## 12.3 ENVIRONMENTAL STRESS AND AGRICULTURE

Abrupt environmental changes have a significant impact on the ecosystem, public health, as well as crop yield (Arunanondchai et al., 2018). The fast expansion of the global population has resulted in a proportionate increase in food consumption. Agricultural productivity is heavily influenced by water supply, poor air quality, and soil nutrients (Noya et al., 2018). In response to



rapid changes in environmental circumstances, severe consequences on plant production are intensifying due to the primary and secondary consequences of abiotic stressors. To quantify the consequences of a climatic emergency, as well as environmental changes, the frequency of stress intervals, its influence on everyday life, and destruction of crop, are used. Plant development and productivity are greatly influenced by abiotic stressors. In their natural habitat, plants are regularly subjected to a range of stressors, including water-logging, rainlessness, high temperature, low temperature, and salt (Ashraf et al., 2018; Benevenuto et al., 2017). Abiotic factors that cause additional stress include UV-B, light intensities, floods, gaseous release, as well as physicochemical variables (Suzuki et al., 2014). The average temperature of the Earth is anticipated to rise from 2°C to 4.5°C in the 21<sup>st</sup> century. As per IPCC-2014 (<http://www.ipcc.ch/>), the period amid the 19<sup>th</sup> and 21<sup>st</sup> centuries are regarded as the most warming (Pachauri et al., 2014). Floods are caused by extreme precipitation events, whereas drought stressors are caused by a lack of rainfall or a complete lack of rainfall over an extended period of time (Khan et al., 2016).

Rice, wheat, and maize are among the world's staple cereal crops satisfying daily food demand (Tack et al., 2015). It is stated that the decrease in the grain filling phase as a result of rising temperatures is the primary cause of crop productivity loss in changing climatic circumstances. As a result, crop yield maintenance and the development of stress-tolerant crop plants are key tasks in modern agriculture (Abhinandan et al., 2018). Plant organs and tissues are harmed by numerous pressures and react consequently. Transcriptional responses to diverse stressors differ in individual root cells or tissues. Salinity, drought, and chemical effluence induce cellular signals such as stress-responsive protein production, excess amounts of related solutes, and higher antioxidant ratios (Dinney et al., 2008). These are referred to as fundamental stresses, and they result in secondary stresses such as oxidative and osmotic stress (Carvalho & Amâncio, 2019).

## **12.4 BASIC OF EPIGENETICS – EPIGENETIC CHANGES AND REQUISITE MACHINERY**

In plants and animals, epigenetic changes, which involve alteration of histones, DNA methylation, chromatin restructuring, histone variants, as well as sRNAs, modification of gene expression are not influenced through DNA sequence, they are transmitted by mitosis and meiosis. They can

change chromatin organization, DNA accessibility, and gene activity, and hence influence a range of molecular processes like gene transcription, DNA replication, repair, and recombination (Allis & Jenuwein, 2016; Lebedeva et al., 2017; Pikaard & Scheid, 2014; Zhang et al., 2018). They regulate growth and development, involving differentiation of cell, regeneration, reproduction, flowering, and aging, as well as acclimating plants to a variety of natural cues like pathogen infection, rainlessness, elevated salt, severe temperature, and heavy metal stressors (Chang et al., 2020; Lebedeva et al., 2017; Lee & Seo, 2018; Pikaard & Scheid, 2014; Ramirez-Prado et al., 2018; Zhang et al., 2018). The various epigenetic alterations, as well as their mechanisms, are detailed here.

### 12.4.1 DNA METHYLATION

DNA methylation is a post-replication alteration where certain cytosine bases get methylated at the 5' site forming m<sup>5</sup>C. Methylation takes place in plants within the base C of “CG,” “CHG,” and “CHH” where H signifies A, C, or T (Law & Jacobsen, 2010). The m<sup>5</sup>C known is as “minor base” which may account for up to 30% of the total amount of m<sup>5</sup>C in plants (Grafi & Ohad, 2013; Vaniushin & Belozerskii, 1959). Along with m<sup>5</sup>C, N6-methyladenine was discovered in mitochondrial DNA in several angiosperms (Burianov et al., 1972; Vanyushin et al., 1971). The most common kind of DNA methylation is cytosine methylation, which involves adding a methyl group to the cytosine ring's fifth carbon (Figure 12.4). Enzymatic substitution of methyl group takes place with S-adenosyl L methionine (SAM) donor by the enzymatic modification whereas non-enzymatic DNA methylation is also evident (Vanyushin, 2014). Cytosine methylation is universally present inside CG dinucleotide of all life forms; but, in plants, both symmetric (CG and CHG) as well as asymmetric (CHH) DNA methylation has been reported (H stands for A, C, or T). Levels of methylation in *Arabidopsis* are 24% in CG, 6.7% in CHG, and 1.7% in CHH sites (Dhar et al., 2014). Because of their symmetry, CG and CHG methylation can be easily repeated during DNA replication, whereas after each replication cycle CHH must undergo *de novo* synthesis. Three types of enzymes are necessary for methylation in plants. These are:

1. **DNA Methyltransferase 1 (MET1):** Primarily engaged in the methylation of symmetric methylation sites for the support of methylation (Sahu et al., 2013).

2. **Chromomethylase 3 (CMT3):** It is a plant-specific enzyme that retains asymmetric regions methylated, like centromeric repeats and transposons (Chan et al., 2005).
3. **Domains Rearranged Methylase (DRM):** It is associated with the asymmetric site for *de novo* methylation (Cao & Jacobsen, 2002). DRM is categorized into two kinds: DRM1 and DRM2. With the use of short interfering RNAs (si-RNAs), DRM2 has been revealed to be concerned with the RNA-directed DNA methylation (RdDM) process (siRNAs) (Hauser et al., 2011).

Whether active or passive, the reversible nature of methylation is aided by demethylation. Removal of an ancestral mark or *de novo* methylation can be avoided during the passive procedure following the replication phase. It occurs when DNMT1 fails to methylate freshly produced DNA strands following several replication cycles after being treated with 5-azacytidine (Sahu et al., 2013). DEMETER (DME), Demeter-Like 2 (DML2), Demeter-Like 3 (DML3), as well as Repressor of Silencing 1 (ROS1) are DNA glycosylases and AP lyases involved in the active process (Grativol et al., 2012). DNA methylation at the promoter of the gene suppresses transcription, but methylation of the gene body can be beneficial or deleterious on gene expression. Genes that are expressed seldom are highly methylated, whereas genes that are expressed frequently are barely methylated (Zemach et al., 2010). *Arabidopsis* DRM2 gene gets methylates both – cytosine residues at CCGG sites, as well as adenines on N6 residues of the GATC locus (Ashapkin et al., 2002). As a result, it is possible to conclude that two distinct forms of modification coexist. Although cytosine methylation is thought to influence adenine methylation, cytosine methylation is thought to influence adenine methylation though their relationship has yet to be discovered. Methylation of cytosine has a key function in the existence and maturation of plants by regulating the transcription procedure, in particular the developmental procedure and also incidences like vernalization and acclimatization to stress (Vyse et al., 2020).

### 12.4.2 HISTONE MODIFICATION

Histone alterations are one of the epigenetic mechanisms that have been most explored and are the most varied. Nucleosome form a constitutional component of chromatin is made up of eight molecules of core histones. Nucleosomes are wrapped with 145–147 bp of double-stranded DNA and

involve 2 molecules of each histone, i.e., H2A, H2B, H3, and H4 (Luger et al., 1997; McGinty & Tan, 2015). Protruding unstructured N-terminal tail of histone is exposed towards diverse post-transcriptional changes which include methylation, acetylation, phosphorylation, ubiquitinylation, glycosylation, ADP-ribosylation, and sumoylation (Figure 12.2) (Sadakierska-Chudy & Filip, 2015). By changing the interface among histones and the adjacent DNA, as well as the interaction of other transcription factors to DNA, can modify chromatin structure and gene transcription (Allis & Jenuwein, 2016; Chang et al., 2020; Pikaard & Scheid, 2014). Methylation and acetylation of histones have been well-described. They play diverse functions of plants, i.e., evolution, development, and reproduction.

Histone acetylation and methylation are two epigenetic markers that are now acknowledged as crucial and ubiquitous in gene regulation (Xu et al., 2017). The enzymes involved in histone modifications are fascinating since some operate as “writers” some are “erasers” while others act as “readers” also known as “effector proteins.” Writer enzymes which include HATs, methyltransferases, kinases, and ubiquitinates catalyze the attachment of chemicals to the histone tail or core domain. On the other hand, eraser enzymes which comprise deacetylases (HDACs), phosphatases, demethylases (HDMs), as well as deubiquitinases remove those alterations caused by writers (Figure 12.3). “Readers” proteins usually offer an exposed surface (such as a crater or surface groove) for a changed histone residue and detect the alteration (acetylation versus methylation) or even the specific state (such as mono-, di- or tri-methylation of lysine). In order to discriminate sequence context, readers contact the neighboring sequence of the changed amino acid. Amidst methyl-lysine-binding motifs discovered in the “reader” proteins are PHD, chromo, Tudor, PWWP, WD40, BAH, ADD, ankyrin repeat, MBT, and zn-CW domains, which may distinguish desired methyl-lysines based on its methylation state and adjacent amino-acid sequence (Xu et al., 2017). Histone acetylation imprints (particularly H3 and H4) improve DNA accessibility by neutralizing the basic charge on histones, which weakens histone and DNA methylation (Allis & Jenuwein, 2016; Onufriev & Schiessel, 2019). In *Arabidopsis*, histone alterations – methylation have both suppressive (symmetric H4R3me2, H3K9me2/3, and H3K27me3) as well as operative (asymmetric H4R3me2, H3K4me3, and H3K36me2/3) mark (Liu et al., 2010; Wang et al., 2016). Histone methylation, unlike acetylation, preserves the electron charge of Lys and does not affect histone proteins’ electrostatic properties. The occupancy or lack of methyl group either on Lys or Arg, or both Lys and Arg amino acids on histones cause

changes in chromatin structure resulting in either transcriptional repression or stimulation (Teperino et al., 2010). ADD (ATRX-DNMT3-DNMT3L), ankyrin, Bromo-adjacent homology, chromo-barrel, chromodomain (CD), double chromodomain, plant homeodomain (PHD), Pro-Trp-Trp-Pro (PWWP), SAWADEE, Tudor, WD40, and zinc finger CW have all been observed to detect either unmethylated and methylated Lys or Arg moiety therefore act as “readers” (Allis & Jenuwein, 2016; Andrews et al., 2016; Bannister & Kouzarides, 2011; Taverna et al., 2007; Xu et al., 2017). The reader proteins mentioned seems to have largely stable across a wide range of eukaryotic species; nevertheless, in *Arabidopsis*, a single Tudor domain of the EMSY-LIKE1 H3K4me2/3 reader protein have been discovered (Zhao et al., 2018). Monoubiquitination of H2A (H2Aub) and H2B (H2Bub) are involved in both an operative as well as suppressive signal in eukaryotic transcription. *Arabidopsis* H2AK121 monoubiquitination (H2AK121ub) co-localizes with H3K27me3 autonomously and never collaboratively with POLYCOMB REPRESSIVE COMPLEX2 (PRC2) a protein that is required for the maintenance of H3K27me3. The function of Polycomb Group (PcG) controls the accessibility of chromatin. At transcriptional regulatory hotspots, H2AK121ub is connected to a less accessible and yet permissive chromatin. Regardless of the fact that the reduction of PcG activity increases chromatin accessibility, this is not always followed by transcriptional activation, suggesting that accessible chromatin is not always a predictor of gene expression (Bratzel et al., 2010; Yin et al., 2021; Zhou et al., 2017). For DNA methylation and heterochromatic histone H3 methylation, deubiquitination of H2B is essential (Sridhar et al., 2007). H2B monoubiquitination particularly stimulates transcription by the accumulation of H3K4me3 (Geng et al., 2012). During the last three decades, multiple studies found turnover rates from fast (few minutes) to sluggish across four core histones, identified in yeast (*Saccharomyces cerevisiae*) to multicellular plants (Waterborg, 2002; Zheng et al., 2013). Due to their longer half-life, methylation may have a benefit of prolonged response, while seen in flowering and the inheritance of transgenerational stress memory (priming). Several research demonstrated numerous epigenetic regulators for acetylation that are involved in the abiotic stress response (Asensi-Fabado et al., 2017; Luo et al., 2017). Histone or/and DNA methylation along with other PTMs like Ser-5P RNAPolII act as epigenetic regulation of prolonged responses like blossoming, stress memory (Ding et al., 2012). Since these epigenetic changes are variable, readers, writers, and erasers must collaborate (Xu et al., 2017). To completely comprehend epigenetic regulation, it is necessary to identify the epigenetic

factors involved in stress response or to elucidate the molecular circuit which they regulate. Understanding how epigenetic regulators are engaged to particular locations in chromatin to perform their function is among the several intriguing features of studying the molecular process involved in the triggering the stress response in plants. The recruitment of epigenetic regulators to specific chromatin regions has been proposed by utilizing: (i) transcription factor (TF) mediated; (ii) long noncoding RNA-mediated; and (iii) self-targeting models (Deng et al., 2018). Recruiters involved in stress response were classified to a TF-mediated model in abiotic stress response. For instance, in rice (*Oryza sativa*) indeterminate spikelet 1 (IDS1) has been recognized as HDAC recruiter and in *Arabidopsis*, MYB96 is identified as the same in case of drought stress (Cheng et al., 2018; Lee & Seo, 2019). During the last few decades, we have gathered more information about the interactions of a variety of epigenetic components found in chromatin, this has improved the knowledge of epigenetic control over abiotic stress. Stress acclimatization can be facilitated by activating or inhibiting gene expression (Tan et al., 2014; Ueda & Seki, 2020; Zhao et al., 2019).

The methylation of histone molecules is limited to lysine and arginine residues at various locations on the molecule (H3, H4). Histone methyltransferase (HMT) consists of histone lysine methyltransferases (HKMTs) and protein arginine methyltransferases, which are responsible for the transfer of methyl to histone (PRMTs) (Liu et al., 2010; Zhao et al., 2019). Besides HMT families, SAM is also a donor of a methyl group to histone for Histone methylation. Histone methylation is classified into three groups: mono-, di-, and tri-methylation based on the number of methyl groups that occur over histone molecules. The various changes that occur on histones have a distinct impact on gene expression (Liu et al., 2010; Zhao et al., 2019). In *A. thaliana* trimethylation of Lys 27 (H3K27me3) suppresses gene expression while H3K4me3 increases gene transcription (Berr et al., 2011; Zheng & Chen, 2011). Histone demethylases (HDMs) are enzymes that remove methyl groups from histones. HDMs are divided into two categories in plants: (i) lysine-specific demethylase 1 (LSD1); and (ii) Jumonji C domain-containing proteins (JMJs) (Peng et al., 2017; Shi et al., 2004). In the case of the two enzymes, the mechanism for demethylation of histone is distinct, as are the cofactors they utilize. JMJs belong to the 2-oxoglutarate-dependent dioxygenase family; on the other hand, LSD1 belongs to the flavin-dependent amine oxidase family (Liu et al., 2010; Xiao et al., 2016). Histone acetylation is a covalent alteration of histone molecules that involves the transfer of acetyl groups ( $\text{CH}_3\text{COO}^-$ ) from acetyl CoA to the  $\epsilon$ -amino group of the

lysine. As a result of this change, the (+) charge on lysine is neutralized, the connection across the altered histone as well as DNA is weakened, allowing the chromatin to unfold (Marmorstein & Zhou, 2014; Zhao et al., 2019). Acetylated histones may engage different proteins that are able to alter chromatin structure (Nie et al., 2019; Zhang et al., 2016). Histone hyperacetylation enhances transcriptional upregulation, and hypoacetylation promotes gene suppression (Marmorstein & Zhou, 2014; Zhang et al., 2016; Zhao et al., 2019). HATs and HDACs have opposing activities that govern histone acetylation levels (Pandey et al., 2002; Pikaard & Scheid, 2014). Plant HATs are classified in four groups: (i) p300/CREB (cAMP-responsive element-binding protein)-binding proteins (CBP); (ii) TATA-binding protein-associated factors (TAF1); (iii) (GNAT) GCN5-related N-terminal acetyltransferases; (iv) MYST (MOZ, Ybf2/Sas3, Sas2, and Tip60-related proteins) (Pandey et al., 2002). HDACs are important epigenetic regulators that suppress gene expression in a range of cellular activities such as plant growth, development, and stress response. Plant's HDACs may be classified into three families: (a) reduced potassium dependency 3/histone deacetylase 1 (RDP3/HDA1); (b) silent information regulator 2 (SIR2); and (c) plant-specific histone deacetylase 2 (PSHDA2) (Yang et al., 2018). The HDACs employ different cofactors: the catalytic domain of SIR2 (sirtuins) proteins requires nicotinic adenine dinucleotide (NAD<sup>+</sup>) as a cofactor; whereas the RPD3/HDA1 family need a Zn<sup>2+</sup> cofactor to deacetylate and the HD2 family proteins are zinc-dependent HDACs since they have a conserved pentapeptide motif (MEFWG) at their N-terminus (Lee & Cho, 2016; RM et al., 2020; Seto & Yoshida, 2014).

### **12.4.3 NON-CODING RNAS**

sRNAs are non-coding RNAs (ncRNAs) with 20–24 nt that are contemporary in the field of epigenetic control. They are small weapons (bio-regulators) that protect plants against diverse stress. ncRNAs are key epigenetic regulators in plants that regulate a variety of cellular activities, like growth, development, or even respond to abiotic stress. ncRNAs are classified into different types depending on their length: (i) non-coding RNAs with a short chain; and (ii) non-coding RNAs with a long chain (Brant & Budak, 2018; Hou et al., 2019; Wang et al., 2017). Several short chain ncRNAs consisting of miRNAs and siRNAs were documented over the years with a role in both transcriptional and post-transcriptional gene regulation in animals and plants

(Brant & Budak, 2018; Hou et al., 2019; Wang et al., 2017). Long ncRNAs (lncRNAs) having transcripts length of > 200 nucleotides. They are involved in chromatin remodeling, transcriptional, post-transcriptional regulation, as well as interact with various RNA molecules via numerous chromatin-mediated processes, rather than being translated into protein.

miRNAs are single-stranded, ncRNAs, which bind to the mRNAs to prevent translation or promote mRNA degradation. miRNA in plants generally has 20–24 nucleotides. They control a wide range of cellular activities, such as root, stem, leaf, and flower development, the transition of vegetative to reproductive phase, as well as adaptive responses to a variety of abiotic and biotic stimuli. RNA polymerase-II transcribes miRNA coding genes to produce pri-miRNAs that are subsequently splitted and transformed by DICER-LIKE (DCLs) proteins to mature miRNAs. In *Arabidopsis*, ARGONAUTE (AGO) protein groups like AGO1 attach to emerging miRNAs then directs target-oriented post-transcriptional gene silencing (PTGS) (Ravi-chandran et al., 2019). siRNAs, although resembling miRNA but in both, RNA polymerase IV (RNAPIV) synthesizes 20–24 nt long straight chain, dsRNAs, through transposons as well as repetitive sequences. Numerous types of siRNAs were discovered to date, many are employed in PTGS, while some are used for TGS (Sun et al., 2013; Yu et al., 2019).

#### **12.4.4 RNA DIRECTED METHYLATION OF DNA**

RNA-directed methylation DNA (RdDM) is a cellular mechanism that involves the insertion of a methyl group on the DNA that takes place in particular DNA sequences that are guided by non-coding RNA molecules. RdDM plays an important role in a variety of cellular activities within plants, comprising stress response, intercellular connections, as well as genomic stability is maintained via transposable element (TE) silencing. RdDM improves plant adaptability towards a range of abiotic stressors, including heat, drought, shortage in phosphate, salt, etc. (Fortes & Gallusci, 2017). The RdDM pathway is specific for plants, which is distinct from the fungi and mammals. The pathway is similar to other sRNAs, especially the RNAi pathway is well-conserved among fungi, plants, and mammals. RdDM and RNAi both generate sRNAs and include the preserved Dicer Argonaute proteins and RNA-dependent RNA polymerase. The addition of methyl group on the DNA by RdDM is typically linked to the transcription repression of the gene. The DNA methylation supplemented by RdDM is typically



linked to the transcription of the pathway-targeted genomic regions. Because plant DNA methylation methods are inherited, such modifications are typically passed on from one generation to its offspring in a stable manner. The most important function of RdDM is to inhibit TE activity in a steady and transgenerational manner. RdDM is a key mechanism in plants that controls a variety of activities by developing and maintaining particular DNA methylation profiles that may have trans-generational epigenetic impacts upon gene expression as well as phenotype (Erdmann & Picard, 2020). When plants are subjected to heat stress, multiple elements connected with the RdDM system are activated, and mutations in specific RdDM machinery components diminish heat tolerance, implying that RdDM plays a key function during heat stress (Liu et al., 2015; Popova et al., 2013). RdDM can regulate genes to produce an effective stress response, in addition to regulating TEs during stress. When the humidity is low, leaves produce fewer stomata as RdDM-mediated downregulation of two genes are involved in stomatal formation (Tricker et al., 2012). Saline stress downregulates of RdDM which has been shown to stimulate the production of a salt stress resistance transcription factor (Xu et al., 2015).

## **12.5 TECHNIQUES USED FOR UNDERSTANDING EPIGENETIC CHANGES IN PLANTS**

Detection of epigenetic changes in plants can be done utilizing a variety of methods; the most well-known of them are discussed in subsections.

### **12.5.1 HISTONE MODIFICATION**

The different post-translational modifications on the protruding amino acids in the N-terminal end of histone proteins are studied by the techniques given in subsections.

#### **12.5.1.1 CHROMATIN IMMUNOPRECIPITATION (CHIP) TECHNIQUE**

ChIP is a technique for determining DNA protein interaction. The technique involves cross-linking of chromatin (DNA-protein) by using mild formaldehyde. The next step is the shearing of genomic DNA into smaller fragments of 200–800 base pairs with the help of either physically (Sonication)

or chemically (Micrococcal nuclease (MNase) enzyme). The protein-DNA complex is precipitated using antibodies produced particularly for the desired protein. Acid treatment is employed for the release of precipitated DNA, which is then detected by either sequencing, microarray or amplified via polymerase chain reaction (PCR) (Park, 2009).

#### 12.5.1.2 CHIP-SEQUENCING (CHIP-SEQ)

ChIP assay coupled with the next-generation sequencing methods is an effective approach for locating the different sites across the whole genome where DNA binds with transcription factors and other proteins. The use of next-generation sequencing (NGS) on ChIP has provided useful data on gene regulatory events involved in a variety of biological processes. ChIP-Seq may be utilized for global mapping of protein-DNA interacting regions genome-wide. Unlike arrays and other epigenome-related methods, which are biased by the use of probes produced from known sequences, ChIP-Seq does not require any previous information. ChIP-Seq uses massively parallel sequencing to do genome-wide profiling, yielding millions of counts across many samples enabling a cost-effective, precise, and impartial examination of epigenetic profile (Park, 2009).

#### 12.5.1.3 CHIP PCR

In this protocol analysis of protein-bound DNA sequences are immunoprecipitated then the DNA fragments are detected and/or quantified in ChIP-PCR with specific primers using TaqMan or Sybr Green Technologies. In this technique, histone modifications and/or protein binding to a known selected of desired loci in the genome are investigated using PCR. ChIP-qPCR allows rapid and quantitative comparisons of a particular area within the genome across numerous samples. This approach is less expensive and faster than whole-genome sequencing.

#### 12.5.1.4 CHIP MICROARRAY (CHIP-CHIP)

ChIP microarray (ChIP-Chip), or genome-wide mapping, integrates chromatin immunoprecipitation (ChIP) with DNA microarray for discovering cellular protein-DNA associations. The antibody pulled down DNA is labeled

and linked to a differently labeled reference specimen as well as used for the analysis of enhanced signals on DNA microarrays. The use of genome tiling microarray technology enables for a whole-genome study of proteins bound to isolated DNA, as well as the construction of a high-resolution genomic map of protein-binding and protein changes. With the help of computational and bioinformatic techniques, the enriched and reference channels are then normalized. The signals are connected with DNA microarray-represented sections within the genome, reliability criteria are provided, as well as maps of protein-genome interaction are generated. The technique may be used to find transcription factor, enhancer, and repressor binding sites, as well as compare these types of bound proteins in normal and treated samples. With the decrease in the cost of NGS significantly and identical findings can be achieved using ChIP-seq, more people are opting for ChIP-seq over ChIP-chip (Ren et al., 2000).

### **12.5.2 PROFILING OF DNA METHYLATION IN PLANTS**

DNA methylation is an epigenetic alteration that regulates gene expression in various activities of organisms. It is accurate for each tissue, active, depends on sequence pattern, as well as inherited across generations, highlighting the need for profiling DNA methylation to address biological issues. DNA methylation is detected by either using next-generation sequencing technology or by microarray hybridization technology.

#### **12.5.2.1 BISULFITE SEQUENCING OF THE WHOLE GENOME (WGBS)**

Next-generation sequencing technology is presently being used to build genomic maps of DNA methylation with single-base clarity. When methylated cytosines are treated with bisulfite then they get converted to uracil. This technique compares the treated specimen to a known specimen to identify the methylated cytosine (Cokus et al., 2008; Kurdyukov & Bullock, 2016). Whole-genome bisulfite sequencing (WGBS) assesses individual cytosines inside a query sequence for almost every cytosine within the genome.

#### **12.5.2.2 METHYLATED DNA IMMUNOPRECIPITATION (MEDIP)**

The disrupted genomic DNA is pulled down with the help of a 5-methylcytosine-specific antibody. PCR based or microarrays of whole genome are used

to evaluate the precipitated DNA (Neary & Carless, 2020; Zhang et al., 2006; Zilberman et al., 2007).

### 12.5.2.3 REDUCED-REPRESENTATION BISULFITE SEQUENCING (RRBS)

Bisulfite sequencing can be used for obtaining single-nucleotide accuracy on DNA methylation inside genomic segments generated by using restriction enzymes. Genetic and epigenetic variations can be compared due to the availability of sequencing and methylation variation within the same set of loci. The technique depends upon MspI restriction digestion, bisulfite conversion and sequencing of (40 and 220 base pairs) digested fragments (Gu et al., 2011). RRBS can be used in the research of plant communities as well as used on plants without any genomic database (Trucchi et al., 2016; van Gurp et al., 2016). RRBS has also been utilized in oak (Platt et al., 2015) and *Brassica rapa* (Chen et al., 2015) populations.

### 12.5.3 SMALL RNA SEQUENCING

Small RNAs (sRNAs) are ncRNA molecules that have a role in gene silencing as well as controls post-transcriptional gene expression. sRNA Sequencing (sRNA-seq) is a technique that allows for in-depth analysis of small RNAs, particularly miRNAs and siRNAs. sRNA-Seq is a next-generation sequencing-based method for miRNA profiling that is presently the most powerful and efficient technique.

## 12.6 CHROMATIN MODIFICATION

The dynamic variation of chromatin architecture in a controlled manner, so that condensed genomic DNA is accessible to regulatory transcription machinery proteins, and therefore regulate gene expression, the process is known as chromatin remodeling. The gene expression occurs only when the chromatin of a cell is “open.” The shift from a repressed to an active state is facilitated by chromatin modification and remodeling machinery. Pioneer transcription factors are the foremost to bind to a “closed” promoter and enhancers, allowing other sequence-specific transcription factors’ easier access to these genomic regions. In conjunction with DNA and histone protein change, several mechanisms such as chromatin assembly/

disassembly, alterations in nucleosome accessibility, nucleosome content, and DNA-protein associations also contribute to transcription regulation as well as other nucleosome and DNA-related alterations (Pikaard & Scheid, 2014). ATP-dependent protein complexes known as chromatin remodeling factors mediate chromatin changes by dissociating or relocating nucleosomes. Three types of chromatin remodeling ATPases have been identified: (i) SWF (switch)/SNF (sucrose non-fermenting) ATPases (Tang et al., 2010); (ii) CHD (chromodomain and helicase-like domain ATPases) (Marfella & Imbalzano, 2007); and (iii) ISWI (imitation switch) ATPases. In yeast, the SWF1/SNF complex is an initial chromatin modification ATPase to be found, they are responsible for mating-type switching (SW1) and sucrose non-fermenting (SNF) deficiencies (Sudarsanam & Winston, 2000). Histone chaperones get involved in histone accumulation as well as elimination in nucleosome formation and dissolution.

### **12.6.1 TRANSGENERATIONALLY INHERITED EPIGENETIC STRESS MEMORY**

In addition to plant genome sequences, epigenome has now been proved to have a role in stress responses. The epigenome is defined as a set of biochemical alterations to DNA sequences or associated proteins that regulate the genome (Bernstein et al., 2007; Park et al., 2016). Epigenetic memory has become a plant's adaptation strategy to a constantly changing world, particularly to severe biotic and abiotic stressors. Not only under stress conditions but also in normal or under mild stress conditions, these epigenetic changes take place in the plants as creating memory these changes, that continues from one generation to the next (Ashapkin et al., 2020). As discussed earlier in the epigenetic regulation, there are three main components of gene regulations such as DNA methylation, modifications of histone, and the expression of small RNAs (sRNAs). Plants often develop a memory of stress exposures at the somatic level, allowing them to better withstand future encounters with similar and different stresses. These mechanisms are known as stress acclimation in the case of abiotic and systemic acquired resistance (SAR) in the case of biotic stress exposure. The acquired memory of stress exposure is also seen in the progenies of plants. This memory can be stored in the chromatin conformation and at the distinct level of sRNAs in the cytoplasm of gametes and growing embryos, priming an organism to cope better with future situations (Kachroo & Robin, 2013). The phenomena of stress-induced

acquired memory are also known as *transgenerational memory*, transgenerational consequences, transgenerational inheritance is described as the ability to pass on information from one generation to the next, the ability of an organism to “remember” its surroundings at the molecular level, resulting in a change in phenotype of the progeny (Tricker, 2015). The term priming was coined to describe how mild stress could induce plant stress responses, allowing them to respond faster and stronger when the stress recurs. In general, being able to recall previous molecular experiences and use this preserved information to adjust to new environments is life-saving when repeated biotic and abiotic challenges occur. The phenomenon of priming of organismal stress responses explains how a plant is modified and gets ready for subsequent stress exposure (a ‘triggering stress cue’) by a temporally restricted environmental stimulation (a “priming stress cue”) (Lämke & Bäurle, 2017). Epigenetic regulation is closely linked to the formation of stress memory (Friedrich et al., 2019; Lämke & Bäurle, 2017). Stress-induced chromatin alterations might influence particular regions or occur genome-wide and are frequently linked to transcriptional regulation. Most of these alterations occur only during the stress exposure, both the expression as well as chromatin states usually return to pre-stress levels quickly. While other modifications cause novel chromatin structures and altered expression of stress-responsive genes, to last longer post-stress exposure prepares an organism for developmental options or even more efficient defense. Plants’ future responses towards stress may be altered by prior exposure to stress, resulting in quicker and/or extreme responses, suggesting the plants have a type of “stress memory.” These epigenetic changes persist or are even transmitted in the next progeny (Pecinka & Mittelsten Scheid, 2012). *Arabidopsis* show transcriptional stress memory after several exposures to drought stress, as evidenced by the rise in transcription rate and increased transcript amount in a group comprising stress-response genes (trainable genes). Accumulation of H3K4me3 on “trainable genes” is more than “non-trainable genes,” implying H3K4me3 can operate like a persistent epigenetic impression linked to transcriptional memory. Trainable genes generate transcripts to the baseline during recovery, i.e., watered condition, however they are linked with unusually high amounts of H3K4me3 along with Ser5P polymerase II, representing the RNA polymerase II is paused (Ding et al., 2012). H3K4me3 and H3K27me3 co-occur but functions independently in the transcription of memory genes of dehydration stress-response. Whereas H3K27me3 does not work as an epigenetic memory imprint of the dehydration stress-responsive genes (Liu et al., 2014). Heat Shock Proteins (HSPs) like HSP21,

HSP22.0, and HSP18.2 having distinct expression levels from Heat Shock-inducible non-memory genes like HSP70 and HSP101, their expression rises shortly following Heat Shock and then rapidly decreases. HSP22.0, a heat stress-induced gene, is implicated in heat stress memory. Heat stress causes the deposition of active histone marks in both the HSP22.0 and HSP70 genes, with H3K4me3, H3K4me2, as well as H3K9Ac. However, once the plants were returned to their natural state, the levels of these three HSP70 markers dropped to baseline levels. HSP22.0, on the other hand, maintained H3K4me3 and me2 levels high, however, H3K9Ac levels remain the same (Lämke et al., 2016). Although several studies have shown that distinct histone modifications mediate transcriptional memory, it is still unknown how adverse conditions cause changes in epigenetic alterations (Berger, 2007; Heard & Martienssen, 2014; Zheng et al., 2017). The generation of transgenerational memory in the offspring, helps the plant to achieve well-balanced survival and reproductive mechanism. DNA methylation in rice takes place due to drought stress and more than 40% of this epimutation are carried to the next progeny. By this time, the tiller number get decreased and showed an increasing rate of seed set. The theory of maintaining a DNA methylation state due to rain-lessness in progeny, aids towards water deficiency stress response as well as prolonged adaptability to water deficiency stress environment. Following the selective pressure of comparatively long-term environmental stress, offspring generations produced a higher balance between “survival strategy” and “reproductive strategy” than the parental generations, resulting in fewer effective tillers and higher seed setting rates than that of the parental generations (Zheng et al., 2017). DNA methylation has been shown to have a role in the transgenerational memory towards heavy metal toxicity on *Oryza sativa*. The PIB subfamily belonging to heavy metal-transporter P-type ATPases (HMAs) plays a function in the absorption and translocation of heavy metals in plants. Level of HMAs increases in relation to heavy metal stressor, as well as transgenerational gene expression memory have been revealed even when heavy metals were removed (Cong et al., 2019) (Table 12.1).

## **12.7 BIOINFORMATICS TOOLS USED IN STUDYING EPIGENETICS OF PLANT STRESS**

Abiotic stressors are among the most significant aspects limiting agricultural output. Understanding the molecular mechanisms of abiotic stressors in

**TABLE 12.1** List Epigenetic Regulators and Their Transcriptional Targets

Types of Stress	Epigenetic Regulators	Transcriptional Targets	Plants	References
Salinity	HDA1	<i>LEA1, SOS1</i>	<i>Oryza sativa</i>	Cheng et al. (2018)
Drought	HDA15, H3/H4ac	<i>ROP6/10/11</i>	<i>Arabidopsis thaliana</i>	Lee & Seo (2019)
Drought	HAT1, H3k7ac	<i>AREB1</i>	<i>Arabidopsis thaliana</i>	Paixão et al. (2019)
Salinity	H2Bub	<i>PTP1, MKP1</i>	<i>Arabidopsis thaliana</i>	Zhou et al. (2017)
Submergence	AG01/4	<i>HR4</i>	<i>Arabidopsis thaliana</i>	Loreti et al. (2020)
Submergence	5-mC, H3K27me3	ANAC017	<i>Arabidopsis thaliana</i>	Bui et al. (2020)
Drought	H3K4me3	<i>RD20/29a, AtGOLS2, ProDH</i>	<i>Arabidopsis thaliana</i>	Kim et al. (2012)
Dehydration	H3K4/27me3	LTP3/4, HIP2.2	<i>Arabidopsis thaliana</i>	Liu et al. (2014)
Salinity	H3K4me3	P5CS1	<i>Arabidopsis thaliana</i>	Feng et al. (2016)
Heat	H3K4me2/3	APX2, HSP18.2	<i>Arabidopsis thaliana</i>	Lämke, Brzezinka, & Bäurle (2016)
Heat	H3K16ac	HSFA3, HSP101	<i>Arabidopsis thaliana</i>	Buszewicz et al. (2016)
Drought	H3K9ac	Water deprivation-responsive genes	<i>Arabidopsis thaliana</i>	Baek et al. (2020)
Heat	H3K27me3	HSFA2	<i>Arabidopsis thaliana</i>	Liu et al. (2019)
Cold	5-mC in promoter	OsOST1 (Os03g0610900)	<i>Oryza sativa</i>	Guo et al. (2019)

plants is a priority in the current period of the continually changing climate. The expansion of -omics techniques has provided significant tools for promoting successful study in this area, allowing researchers to investigate a growing variety of species, tolerant, and susceptible genotypes. These are comprehensive, multidimensional methods depending on molecular analysis on the genomes, transcriptomes, proteomes, and metabolomes platform are at the present possible, allowing researchers to better understand important molecular components of abiotic stress responses. Bioinformatics is now essential in information generator, excavator, as well as incorporator, to



bring out important data as well as comparing attempts, opening the method for the modeling of the processes concerned.

There are public internet-based information hubs and platforms which are available devoted to plant's reactions to stress. They are important for organizing results and other relevant data from diverse levels of research studies and making it available to all members of the scientific community who are interested.

At the genomic level, some databases are available dedicated to plant response to diverse stress. Plant stress (<https://plantstress.com/>) is a public internet-based information facility, a consultancy service, as well as a platform for an expert update on relevant topics in plant environmental abiotic stress. Plant Stress Gene Database (<http://ccbb.jnu.ac.in/stressgenes/>) is another resource of Plant Stress Gene Database which contains 259 stress-associated genes from 11 species, as well as the entire accessible data on each gene. The database even contains orthologs and paralogs of proteins encoding stress-related genes.

Globally drought is one of the most significant abiotic stressors. Plants have a variety of physiological and molecular drought escaping and drought tolerance strategies to deal with reduced water supply. Several drought stress-related genes determine transcription factors that influence the expression of other genes concerned with a variety of physiological as well as molecular responses under drought stress. DroughtDB (<https://pgsb.helmholtz-muenchen.de/droughtdb/>) is a database that contains curated genes implicated in drought stress response, as well as extensive information on calculated ortholog genes in nine model and crop plants (Alter et al., 2015). PASmiR ([https://tools4mirs.org/software/mirna\\_databases/pasmir/](https://tools4mirs.org/software/mirna_databases/pasmir/)) is a resource on miRNA molecular control for plant abiotic stress based on over 200 existing literature, resulting in 1,038 regulatory connections among 682 miRNAs as well as 35 distinct forms on abiotic stressors in 33 different plant species. The search engine permits users to search for miRNAs by name, species, or kind of abiotic stress (Zhang et al., 2013).

At the proteomic level, some databases are available dedicated to plant response to diverse stress. The plant stress protein database (PSPDB) (<http://bioclues.org/pspdb/>) is a general resource at the protein level that includes 2,064 carefully edited plant stress proteins representing 134 plant species, having functions across 30 distinct types both biotic and abiotic stressors highlighted. One can get information through gene, species, keyword, citation, gene families, as well as taxonomic categorization from the database. PlantPReS ([www.proteome.ir](http://www.proteome.ir)) is another resource of proteomics on plant's

reactions towards biotic and abiotic stressors that has over 20,413 items across 456 carefully selected articles and over 10,600 distinct stress-induced proteins. Since the interface and the analytical tools, such as the query engine, gene ontology, cross-referencing, as well as expression impressions on desired proteins implicated for stressors are user-friendly, so the Plant-PReS is an important facility of the plant stress society (Mousavi et al., 2016; Sobhanian et al., 2020).

The stress responsive transcription factor database (STIFDB v.2) (<http://caps.ncbs.res.in/stifdb2/>) has grown to a huge information hub related to *Arabidopsis thaliana* abiotic stress-responsive genes that may be used to find probable Transcription Factor Binding region on their promoters. HMM-based models are used to identify the binding sites of transcription factors of 10 different families of transcription factors. The curators cross-check the method's probable Transcription Factor Binding Sites to published research reports (Naika et al., 2013). There is currently no metabolomics database available that are primarily focused on abiotic stressors.

## 12.8 CONCLUSION AND FUTURE PROSPECTS

Plants suffer a range of alterations to adapt to their environment because they are subjected to continuously fluctuating natural circumstances. In the last two decades, the mechanism of epigenetic inheritance, as well as its importance, has been investigated a lot in plants, concerning abiotic stress. Although there are still opportunities for understanding epigenetics to promote novel and better approaches to crop development. Histone modification along with the chromatin remodeling, i.e., the open (accessible for transcription) or closed (concealed for transcription) configuration can regulate expression of a gene either positively or negatively, respectively. DNA methylation regulates transcription by engaging proteins occupied during inhibition of gene expression or blocking transcription factor(s) from interacting with DNA. Plant sRNAs usually carry out their biological functions by inhibiting specific targets. The different molecular tools of epigenetics are undoubtedly essential in understanding and controlling abiotic stress response in plants. These tools can also be utilized in regulating stress tolerance and the production of stress-resistant crop plants. The journey of epigenetics started way back in 1942 when Conrad Hal Waddington coined the term. Epigenetics is a huge mountain of information. To date, only a few pebbles have been unearthed thanks to modern technology and the devotion of researchers. With improved

technology and the participation of more researchers, numerous fundamental phenomena of plant metabolism and stress response will undoubtedly be dug out in the near future. How do stresses produce chromatin alterations and subsequent transcriptional modifications via regulating epigenetic machinery Reprogramming is a difficult concept to grasp? It's unclear how stress affects epigenetic machinery, causing chromatin alterations and subsequent transcriptional reprogramming. It's also unclear how stress-related epigenetic modifications might be passed down to progeny as a means of stress retention. Without a doubt, future research should focus on decoding the secret language of abiotic stress in plants impacting epigenetics. The epigenomes of a growing number of plants that are exploited, thanks to rapid advances in automated large-scale sequencing as well as diverse chromatin profiling machinery that could immensely expand the number of research findings on plants' reshaping in response to stress utilizing epigenetic.

## KEYWORDS

- **abiotic stress**
- **DNA methylation**
- **epigenetics**
- **histone modification**
- **post-translational modification**
- **reactive oxygen species**
- **small RNA**

## REFERENCES

- Abhinandan, K., Skori, L., Stanic, M., Hickerson, N., Jamshed, M., & Samuel, M. A., (2018). Abiotic stress signaling in wheat—an inclusive overview of hormonal interactions during abiotic stress responses in wheat. *Front. Plant Sci.*, 9, 734.
- Aina, R., Sgorbati, S., Santagostino, A., Labra, M., Ghiani, A., & Citterio, S., (2004). Specific hypomethylation of DNA is induced by heavy metals in white clover and industrial hemp. *Physiol. Plant.*, 121(3), 472–480.
- Allis, C. D., & Jenuwein, T., (2016). The molecular hallmarks of epigenetic control. *Nat. Rev. Genet.*, 17(8), 487.

- Alter, S., Bader, K. C., Spannagl, M., Wang, Y., Bauer, E., Schön, C. C., & Mayer, K. F., (2015). DroughtDB: An expert-curated compilation of plant drought stress genes and their homologs in nine species. *Database*, 2015, bav046.
- Andrews, F. H., Strahl, B. D., & Kutateladze, T. G., (2016). Insights into newly discovered marks and readers of epigenetic information. *Nat. Chem. Biol.*, 12(9), 662–668.
- Arunanondchai, P., Fei, C., Fisher, A., McCarl, B. A., Wang, W., & Yang, Y., (2018). How does climate change affect agriculture. In: Cramer, G. L., Paudel, K. P., & Schmitz, A., (eds.), *The Routledge Handbook of Agricultural Economics* (p. 191). Routledge.
- Asensi-Fabado, M. A., Amtmann, A., & Perrella, G., (2017). Plant responses to abiotic stress: The chromatin context of transcriptional regulation. *Biochim. Biophys. Acta Gene Regul. Mech.*, 1860(1), 106–122.
- Ashapkin, V. V., Kutueva, L. I., & Vanyushin, B. F., (2002). The gene for domains rearranged methyltransferase (DRM2) in *Arabidopsis thaliana* plants is methylated at both cytosine and adenine residues. *FEBS Lett.*, 532(3), 367–372.
- Ashapkin, V. V., Kutueva, L. I., Aleksandrushkina, N. I., & Vanyushin, B. F., (2020). Epigenetic mechanisms of plant adaptation to biotic and abiotic stresses. *Int. J. Mol. Sci.*, 21(20), 7457.
- Ashraf, M. A., Akbar, A., Askari, S. H., Iqbal, M., Rasheed, R., & Hussain, I., (2018). Recent advances in abiotic stress tolerance of plants through chemical priming: An overview. In: Rakshit, A., & Singh, H., (eds.), *Advances in Seed Priming* (pp. 51–79). Springer, Singapore. [https://doi.org/10.1007/978-981-13-0032-5\\_4](https://doi.org/10.1007/978-981-13-0032-5_4).
- Avramova, Z., (2011). Epigenetic regulatory mechanisms in plants. In: Tollefsbol, T., (ed.), *Handbook of Epigenetics* (pp. 251–278). Academic Press. <https://doi.org/10.1016/C2009-0-30488-2>.
- Azhar, I., Ghosh, S., Adhikari, A., Adhikari, S., Roy, D., Shaw, A. K., Singh, K., & Hossain, Z., (2020). Comparative analysis of maize root sRNA transcriptome unveils the regulatory roles of miRNAs in submergence stress response mechanism. *Environ. Exp. Bot.*, 171, 103924.
- Back, D., Jiang, J., Chung, J. S., Wang, B., Chen, J., Xin, Z., & Shi, H., (2011). Regulated AtHKT1 gene expression by a distal enhancer element and DNA methylation in the promoter plays an important role in salt tolerance. *Plant Cell Physiol.*, 52(1), 149–161.
- Back, D., Shin, G., Kim, M. C., Shen, M., Lee, S. Y., & Yun, D. J., (2020). Histone deacetylase HDA9 with ABI4 contributes to abscisic acid homeostasis in drought stress response. *Front. Plant Sci.*, 11, 143.
- Bailey-Serres, J., & Voesenek, L., (2008). Flooding stress: Acclimations and genetic diversity. *Annu. Rev. Plant Biol.*, 59, 313–339.
- Bannister, A. J., & Kouzarides, T., (2011). Regulation of chromatin by histone modifications. *Cell Res.*, 21(3), 381–395.
- Barrett-Lennard, E., (2003). The interaction between waterlogging and salinity in higher plants: Causes, consequences and implications. *Plant and Soil*, 253(1), 35–54.
- Benevenuto, R. F., Agapito-Tenfen, S. Z., Vilperte, V., Wikmark, O. G., Van, R. P. J., & Nodari, R. O., (2017). Molecular responses of genetically modified maize to abiotic stresses as determined through proteomic and metabolomic analyses. *PLoS One*, 12(2), e0173069.
- Berger, S. L., (2007). The complex language of chromatin regulation during transcription. *Nature*, 447(7143), 407–412.
- Bernstein, B. E., Meissner, A., & Lander, E. S., (2007). The mammalian epigenome. *Cell*, 128(4), 669–681.

- Berr, A., Shafiq, S., & Shen, W. H., (2011). Histone modifications in transcriptional activation during plant development. *Biochim. Biophys. Acta Gene. Regul. Mech.*, 1809(10), 567–576.
- Bewick, A. J., Ji, L., Niederhuth, C. E., Willing, E. M., Hofmeister, B. T., Shi, X., Wang, L., et al., (2016). On the origin and evolutionary consequences of gene body DNA methylation. *PNAS.*, 113(32), 9111–9116.
- Bhadouriya, S. L., Mehrotra, S., Basantani, M. K., Loake, G. J., & Mehrotra, R., (2020). Role of chromatin architecture in plant stress responses: An update. *Front Plant Sci.*, 11, 603380.
- Bird, A., (2007). Perceptions of epigenetics. *Nature*, 447(7143), 396.
- Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I., & Martínez-Estévez, M., (2017). Aluminum, a friend or foe of higher plants in acid soils. *Front Plant Sci.*, 8, 1767.
- Bornman, J. F., (1991). UV radiation as an environmental stress in plants. *J. Photochem. Photobiol. B.*, 8(3), 337.
- Boyer, J. S., (1982). Plant productivity and environment. *Science*, 218(4571), 443–448.
- Brant, E. J., & Budak, H., (2018). Plant small non-coding RNAs and their roles in biotic stresses. *Front Plant Sci.*, 9, 1038.
- Bratzel, F., López-Torrejón, G., Koch, M., Del Pozo, J. C., & Calonje, M., (2010). Keeping cell identity in *Arabidopsis* requires PRC1 RING-finger homologs that catalyze H2A monoubiquitination. *Curr. Biol.*, 20(20), 1853–1859.
- Bui, L. T., Shukla, V., Giorgi, F. M., Trivellini, A., Perata, P., Licausi, F., & Giuntoli, B., (2020). *Differential Submergence Tolerance Between Juvenile and Adult Arabidopsis Plants Involves the ANAC017 Transcription Factor*. bioRxiv 02.12.945923. <https://doi.org/10.1101/2020.02.12.945923>.
- Burianov, I., Eroshina, N., & Vagabova, L., (1972). *In On the Detection of 6-Methylaminopurine in DNA of Higher Plant Pollen* (Vol. 206, pp. 992–994). Akad Nauk SSSR Dokl.
- Buszewicz, D., Archacki, R., Palusiński, A., Kotliński, M., Fogtman, A., Iwanicka-Nowicka, R., Sosnowska, K., et al., (2016). HD2C histone deacetylase and a SWI/SNF chromatin remodeling complex interact and both are involved in mediating the heat stress response in *Arabidopsis*. *Plant Cell Environ.*, 39(10), 2108–2122.
- Cao, X., & Jacobsen, S. E., (2002). Role of the *Arabidopsis* DRM methyltransferases in de novo DNA methylation and gene silencing. *Curr. Biol.*, 12(13), 1138–1144.
- Cao, X., Wu, Z., Jiang, F., Zhou, R., & Yang, Z., (2014). Identification of chilling stress-responsive tomato microRNAs and their target genes by high-throughput sequencing and degradome analysis. *BMC Genomics*, 15(1), 1–16.
- Carvalho, L. C., & Amâncio, S., (2019). Cutting the gordian knot of abiotic stress in grapevine: From the test tube to climate change adaptation. *Physiol. Plant.*, 165(2), 330–342.
- Chan, S. W. L., Henderson, I. R., & Jacobsen, S. E., (2005). Gardening the genome: DNA methylation in *Arabidopsis thaliana*. *Nat. Rev. Genet.*, 6(5), 351–360.
- Chang, Y. N., Zhu, C., Jiang, J., Zhang, H., Zhu, J. K., & Duan, C. G., (2020). Epigenetic regulation in plant abiotic stress responses. *J. Integr. Plant Biol.*, 62(5), 563–580.
- Chen, L. T., & Wu, K., (2010). Role of histone deacetylases HDA6 and HDA19 in ABA and abiotic stress response. *Plant Signal. Behav.*, 5(10), 1318–1320.
- Chen, X., Ge, X., Wang, J., Tan, C., King, G. J., & Liu, K., (2015). Genome-wide DNA methylation profiling by modified reduced representation bisulfite sequencing in *Brassica rapa* suggests that epigenetic modifications play a key role in polyploid genome evolution. *Front. Plant Sci.*, 6, 836.

- Cheng, X., Zhang, S., Tao, W., Zhang, X., Liu, J., Sun, J., Zhang, H., et al., (2018). INDETERMINATE SPIKELET1 recruits histone deacetylase and a transcriptional repression complex to regulate rice salt tolerance. *Plant Physiol.*, 178(2), 824–837.
- Chew, Y. H., & Halliday, K. J., (2011). A stress-free walk from *Arabidopsis* to crops. *Curr. Opin. Biotechnol.*, 22(2), 281–286.
- Chinnusamy, V., & Zhu, J. K., (2009). Epigenetic regulation of stress responses in plants. *Curr. Opin. Plant Biol.*, 12(2), 133–139.
- Chinnusamy, V., Zhu, J., & Zhu, J. K., (2006). Gene regulation during cold acclimation in plants. *Physiol. Plant.*, 126(1), 52–61.
- Cokus, S. J., Feng, S., Zhang, X., Chen, Z., Merriman, B., Haudenschild, C. D., Pradhan, S., et al., (2008). Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature*, 452(7184), 215–219.
- Cong, W., Miao, Y., Xu, L., Zhang, Y., Yuan, C., Wang, J., Zhuang, T., et al., (2019). Transgenerational memory of gene expression changes induced by heavy metal stress in rice (*Oryza sativa* L.). *BMC Plant Biol.*, 19(1), 1–14.
- Deans, C., & Maggert, K. A., (2015). What do you mean, “epigenetic”? *Genetics*, 199(4), 887–896.
- Demirel, U., Morris, W. L., Ducreux, L. J., Yavuz, C., Asim, A., Tindas, I., Campbell, R., et al., (2020). Physiological, biochemical, and transcriptional responses to single and combined abiotic stress in stress-tolerant and stress-sensitive potato genotypes. *Front. Plant Sci.*, 11, 169.
- Deng, X., Qiu, Q., He, K., & Cao, X., (2018). The seekers: How epigenetic modifying enzymes find their hidden genomic targets in *Arabidopsis*. *Curr. Opin. Plant Biol.*, 45, 75–81.
- Dhar, M. K., Vishal, P., Sharma, R., & Kaul, S., (2014). Epigenetic dynamics: Role of epimarks and underlying machinery in plants exposed to abiotic stress. *Int. J. Genomics.*, 2014. <https://doi.org/10.1155/2014/187146>.
- Ding, Y., Fromm, M., & Avramova, Z., (2012). Multiple exposures to drought ‘train’ transcriptional responses in *Arabidopsis*. *Nat. Commun.*, 3(1), 1–9.
- Ding, Y., Shi, Y., & Yang, S., (2019). Advances and challenges in uncovering cold tolerance regulatory mechanisms in plants. *New Phytol.*, 222(4), 1690–1704.
- Dinneny, J. R., Long, T. A., Wang, J. Y., Jung, J. W., Mace, D., Pointer, S., Barron, C., et al., (2008). Cell identity mediates the response of *Arabidopsis* roots to abiotic stress. *Science*, 320(5878), 942–945.
- Driedonks, N., Xu, J., Peters, J. L., Park, S., & Rieu, I., (2015). Multi-level interactions between heat shock factors, heat shock proteins, and the redox system regulate acclimation to heat. *Front. Plant Sci.*, 6, 999.
- Dubouzet, J. G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E. G., Miura, S., Seki, M., et al., (2003). OsDREB genes in rice, *Oryza sativa* L. encode transcription activators that function in drought-, high-salt-and cold-responsive gene expression. *Plant J.*, 33(4), 751–763.
- Ebrahim, S., (2012). Epigenetics: The next big thing. *Int. J. Epidemiol.*, 41(1), 1–3.
- Erdmann, R. M., & Picard, C. L., (2020). RNA-directed DNA methylation. *PLoS Genet.*, 16(10), e1009034.
- Feng, S., Jacobsen, S. E., & Reik, W., (2010). Epigenetic reprogramming in plant and animal development. *Science*, 330(6004), 622–627.
- Feng, X. J., Li, J. R., Qi, S. L., Lin, Q. F., Jin, J. B., & Hua, X. J., (2016). Light affects salt stress-induced transcriptional memory of P5CS1 in *Arabidopsis*. *PNAS*, 113(51), E8335–E8343.

- Flowers, T., (2004). Improving crop salt tolerance. *J. Exp. Bot.*, 55(396), 307–319.
- Fortes, A. M., & Gallusci, P., (2017). Plant stress responses and phenotypic plasticity in the epigenomics era: Perspectives on the grapevine scenario, a model for perennial crop plants. *Front. Plant Sci.*, 8, 82.
- Friedrich, T., Faivre, L., Bäurle, I., & Schubert, D., (2019). Chromatin-based mechanisms of temperature memory in plants. *Plant Cell Environ.*, 42(3), 762–770.
- Fryzova, R., Pohanka, M., Martinkova, P., Cihlarova, H., Brtnicky, M., Hladky, J., & Kynicky, J., (2017). Oxidative stress and heavy metals in plants. *Rev. Environ. Contam. T.*, 245, 129–156.
- Gallo-Franco, J. J., Sosa, C. C., Ghneim-Herrera, T., & Quimbaya, M., (2020). Epigenetic control of plant response to heavy metal stress: A new view on aluminum tolerance. *Front. Plant Sci.*, 11.
- Gaspar, T., Franck, T., Bisbis, B., Kevers, C., Jouve, L., Hausman, J. F., & Dommes, J., (2002). Concepts in plant stress physiology. Application to plant tissue cultures. *Plant Growth Regul.*, 37(3), 263–285.
- Geng, F., Wenzel, S., & Tansey, W. P., (2012). Ubiquitin and proteasomes in transcription. *Annu. Rev. Biochem.*, 81, 177–201.
- Giuntoli, B., Shukla, V., Maggiorelli, F., Giorgi, F. M., Lombardi, L., Perata, P., & Licausi, F., (2017). Age-dependent regulation of ERF-VII transcription factor activity in *Arabidopsis thaliana*. *Plant Cell Environ.*, 40(10), 2333–2346.
- Grafi, G., & Ohad, N., (2013). Plant epigenetics: A historical perspective. In: Grafi, G., & Ohad, N., (eds.), *Epigenetic Memory and Control in Plants* (pp. 1–19). Springer: Berlin Heidelberg.
- Grant-Downton, R., & Dickinson, H., (2005). Epigenetics and its implications for plant biology. 1. The epigenetic network in plants. *Ann. Bot.*, 96(7), 1143–1164.
- Grativol, C., Hemerly, A. S., & Ferreira, P. C. G., (2012). Genetic and epigenetic regulation of stress responses in natural plant populations. *Biochim. Biophys. Acta Gene Regul. Mech. BBA-Gene. Regul. Mech.*, 1819(2), 176–185.
- Gravot, A., Richard, G., Lime, T., Lemarié, S., Jubault, M., Lariagon, C., Lemoine, J., et al., (2016). Hypoxia response in *Arabidopsis* roots infected by *Plasmodiophora brassicae* supports the development of clubroot. *BMC Plant Biol.*, 16(1), 1–10.
- Gu, H., Smith, Z. D., Bock, C., Boyle, P., Gnirke, A., & Meissner, A., (2011). Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. *Nat. Protoc.*, 6(4), 468–481.
- Gull, A., Lone, A. A., & Wani, N. U. I., (2019). Biotic and abiotic stresses in plants. In: De Oliveira, A. B., (ed.), *Abiotic and Biotic Stress in Plants* (pp. 1–19). Intech Open.
- Gulli, M., Marchi, L., Fragni, R., Buschini, A., & Visioli, G., (2018). Epigenetic modifications preserve the hyperaccumulator *Noccaea caerulea* from Ni geno-toxicity. *Environ. Mol. Mutagen.*, 59(6), 464–475.
- Guo, H., Wu, T., Li, S., He, Q., Yang, Z., Zhang, W., Gan, Y., et al., (2019). The methylation patterns and transcriptional responses to chilling stress at the seedling stage in rice. *Int. J. Mol. Sci.*, 20(20), 5089.
- Guo, X., Liu, D., & Chong, K., (2018). Cold signaling in plants: Insights into mechanisms and regulation. *J. Integr. Plant Biol.*, 60(9), 745–756.
- Gupta, B., & Huang, B., (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *Int. J. Genomics*, 2014.

- Hauser, M. T., Aufsatz, W., Jonak, C., & Luschnig, C., (2011). Transgenerational epigenetic inheritance in plants. *Biochim. Biophys. Acta Gene. Regul. Mech.*, 1809(8), 459–468.
- Heard, E., & Martienssen, R. A., (2014). Transgenerational epigenetic inheritance: Myths and mechanisms. *Cell*, 157(1), 95–109.
- Holoch, D., & Moazed, D., (2015). RNA-mediated epigenetic regulation of gene expression. *Nat. Rev. Genet.*, 16(2), 71–84.
- Hou, J., Lu, D., Mason, A. S., Li, B., Xiao, M., An, S., & Fu, D., (2019). Non-coding RNAs and transposable elements in plant genomes: Emergence, regulatory mechanisms and roles in plant development and stress responses. *Planta*, 250(1), 23–40.
- Hsu, F. C., Chou, M. Y., Chou, S. J., Li, Y. R., Peng, H. P., & Shih, M. C., (2013). Submergence confers immunity mediated by the WRKY22 transcription factor in *Arabidopsis*. *Plant Cell*, 25(7), 2699–2713.
- Huang, S. Q., Xiang, A. L., Che, L. L., Chen, S., Li, H., Song, J. B., & Yang, Z. M., (2010). A set of miRNAs from *Brassica napus* in response to sulphate deficiency and cadmium stress. *Plant Biotechnol. J.*, 8(8), 887–899.
- Isayenkov, S. V., & Maathuis, F. J., (2019). Plant salinity stress: Many unanswered questions remain. *Front. Plant Sci.*, 10, 80.
- Jablunka, E., & Lamb, M. J., (2002). The changing concept of epigenetics. *Ann. N. Y. Acad. Sci.*, 981(1), 82–96.
- Jaglo, K. R., Kleff, S., Amundsen, K. L., Zhang, X., Haake, V., Zhang, J. Z., Deits, T., & Thomashow, M. F., (2001). Components of the *Arabidopsis* C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. *Plant Physiol.*, 127(3), 910–917.
- Jaleel, C. A., Gopi, R., Sankar, B., Gomathinayagam, M., & Panneerselvam, R., (2008). Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *Comptes Rendus Biologies*, 331(1), 42–47.
- Jaleel, C. A., Manivannan, P., Sankar, B., Kishorekumar, A., Gopi, R., Somasundaram, R., & Panneerselvam, R., (2007). Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation. *Colloids Surf. B*, 60(1), 110–116.
- James, R. A., Blake, C., Byrt, C. S., & Munns, R., (2011). Major genes for Na<sup>+</sup> exclusion, Nax1 and Nax2 (wheat HKT1; 4 and HKT1; 5), decrease Na<sup>+</sup> accumulation in bread wheat leaves under saline and waterlogged conditions. *J. Exp. Bot.*, 62(8), 2939–2947.
- Jeong, D. H., & Green, P. J., (2013). The role of rice microRNAs in abiotic stress responses. *J. Plant Biol.*, 56(4), 187–197.
- Kachroo, A., & Robin, G. P., (2013). Systemic signaling during plant defense. *Curr. Opin. Plant Biol.*, 16(4), 527–533.
- Kalisz, S., & Purugganan, M. D., (2004). Epialleles via DNA methylation: Consequences for plant evolution. *Trends Ecol. Evol.*, 19(6), 309–314.
- Kaplan, F., Kopka, J., Haskell, D. W., Zhao, W., Schiller, K. C., Gatzke, N., Sung, D. Y., & Guy, C. L., (2004). Exploring the temperature-stress metabolome of *Arabidopsis*. *Plant Physiol.*, 136(4), 4159–4168.
- Khan, A., Ijaz, M., Muhammad, J., Goheer, A., Akbar, G., & Adnan, M., (2016). Climate change implications for wheat crop in Dera Ismail Khan District of Khyber Pakhtunkhwa. *Pakistan Journal of Meteorology*, 13(25).
- Kim, J. H., (2021). Multifaceted chromatin structure and transcription changes in plant stress response. *Int. J. Mol. Sci.*, 22(4).



- Kim, J. M., Sasaki, T., Ueda, M., Sako, K., & Seki, M., (2015). Chromatin changes in response to drought, salinity, heat, and cold stresses in plants. *Front. Plant Sci.*, 6, 114.
- Kim, J. M., To, T. K., Ishida, J., Matsui, A., Kimura, H., & Seki, M., (2012). Transition of chromatin status during the process of recovery from drought stress in *Arabidopsis thaliana*. *Plant Cell Physiol.*, 53(5), 847–856.
- Kim, Y. K., Chae, S., Oh, N. I., Nguyen, N. H., & Cheong, J. J., (2020). Recurrent drought conditions enhance the induction of drought stress memory genes in *Glycine max* L. *Front. Genet.*, 11, 1248.
- Kochian, L. V., Piñeros, M. A., Liu, J., & Magalhaes, J. V., (2015). Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. *Annu. Rev. Plant Biol.*, 66, 571–598.
- Kong, L., Liu, Y., Wang, X., & Chang, C., (2020). Insight into the role of epigenetic processes in abiotic and biotic stress response in wheat and barley. *Int. J. Mol. Sci.*, 21(4), 1480.
- Kotagiri, D., & Kolluru, V. C., (2017). Effect of salinity stress on the morphology and physiology of five different *Coleus* species. *Biomed. Pharmacol.*, 10(4), 1639–1649.
- Kouzarides, T., (2007). Chromatin modifications and their function. *Cell*, 128(4), 693–705.
- Kovalchuk, O., Burke, P., Arkhipov, A., Kuchma, N., James, S. J., Kovalchuk, I., & Pogribny, I., (2003). Genome hypermethylation in *Pinus silvestris* of chernobyl—A mechanism for radiation adaptation? *Mutat. Res-Fund Mol. M.*, 529(1, 2), 13–20.
- Kumar, S., Beena, A. S., Awana, M., & Singh, A., (2017). Salt-induced tissue-specific cytosine methylation downregulates expression of HKT genes in contrasting wheat (*Triticum aestivum* L.) genotypes. *DNA Cell Biol.*, 36(4), 283–294.
- Kurdyukov, S., & Bullock, M., (2016). DNA methylation analysis: Choosing the right method. *Biology (Basel)*, 5(1).
- Kwon, C. S., Lee, D., Choi, G., & Chung, W. I., (2009). Histone occupancy-dependent and-independent removal of H3K27 trimethylation at cold-responsive genes in *Arabidopsis*. *Plant J.*, 60(1), 112–121.
- Lämke, J., & Bäurle, I., (2017). Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. *Genome Biol.*, 18(1), 1–11.
- Lämke, J., Brzezinka, K., & Bäurle, I., (2016). HSFA2 orchestrates transcriptional dynamics after heat stress in *Arabidopsis thaliana*. *Transcription*, 7(4), 111–114.
- Lämke, J., Brzezinka, K., Altmann, S., & Bäurle, I., (2016). A hit-and-run heat shock factor governs sustained histone methylation and transcriptional stress memory. *EMBO J.*, 35(2), 162–175.
- Lang-Mladek, C., Popova, O., Kiok, K., Berlinger, M., Rakic, B., Aufsatz, W., Jonak, C., et al., (2010). Transgenerational inheritance and resetting of stress-induced loss of epigenetic gene silencing in *Arabidopsis*. *Mol. Plant.*, 3(3), 594–602.
- Law, J. A., & Jacobsen, S. E., (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet.*, 11(3), 204–220.
- Lebedeva, M., Tvorogova, V., & Tikhodeyev, O., (2017). Epigenetic mechanisms and their role in plant development. *Russ. J. Genet.*, 53(10), 1057–1071.
- Lee, H. G., & Seo, P. J., (2019). MYB96 recruits the HDA15 protein to suppress negative regulators of ABA signaling in *Arabidopsis*. *Nat. Commun.*, 10(1), 1713.
- Lee, K., & Seo, P. J., (2018). Dynamic epigenetic changes during plant regeneration. *Trends Plant Sci.*, 23(3), 235–247.

- Lee, W. K., & Cho, M. H., (2016). Telomere-binding protein regulates the chromosome ends through the interaction with histone deacetylases in *Arabidopsis thaliana*. *Nucleic Acids Res.*, 44(10), 4610–4624.
- Lichtenthaler, H. K., (1998). The stress concept in plants: An introduction. *Ann. N Y Acad. Sci.*, 851, 187–198.
- Liu, C., Lu, F., Cui, X., & Cao, X., (2010). Histone methylation in higher plants. *Annu. Rev. Plant Biol.*, 61, 395–420.
- Liu, J., Feng, L., Gu, X., Deng, X., Qiu, Q., Li, Q., Zhang, Y., et al., (2019). An H3K27me3 demethylase-HSFA2 regulatory loop orchestrates transgenerational thermomemory in *Arabidopsis*. *Cell Res.*, 29(5), 379–390.
- Liu, J., Feng, L., Li, J., & He, Z., (2015). Genetic and epigenetic control of plant heat responses. *Front Plant Sci.*, 6, 267.
- Liu, X., Shi, Y., & Yang, S., (2018). Insights into the regulation of C-repeat binding factors in plant cold signaling. *J. Integr. Plant Biol.*, 60(9), 780–795.
- Liu, L., Yin, H., Liu, Y., Shen, L., Yang, X., Zhang, D., Li, M., & Yan, M., (2021). Analysis of cadmium-stress-induced microRNAs and their targets reveals bra-miR172b-3p as a potential Cd<sup>2+</sup>-specific resistance factor in *Brassica juncea*. *Processes*, 9(7), 1099.
- Liu, N., Ding, Y., Fromm, M., & Avramova, Z., (2014). Different gene-specific mechanisms determine the ‘revised-response’ memory transcription patterns of a subset of *A. thaliana* dehydration stress responding genes. *Nucleic Acids Res.*, 42(9), 5556–5566.
- Liu, N., Fromm, M., & Avramova, Z., (2014). H3K27me3 and H3K4me3 chromatin environment at super-induced dehydration stress memory genes of *Arabidopsis thaliana*. *Mol. Plant.*, 7(3), 502–513.
- Liu, Y., Dang, P., Liu, L., & He, C., (2019). Cold acclimation by the CBF–COR pathway in a changing climate: Lessons from *Arabidopsis thaliana*. *Plant Cell Rep.*, 38(5), 511–519.
- Lobell, D. B., & Gourdji, S. M., (2012). The influence of climate change on global crop productivity. *Plant Physiol.*, 160(4), 1686–1697.
- Lobell, D. B., Schlenker, W., & Costa-Roberts, J., (2011). Climate trends and global crop production since 1980. *Science*, 333(6042), 616–620.
- Loreti, E., Betti, F., Ladera-Carmona, M. J., Fontana, F., Novi, G., Valeri, M. C., & Perata, P., (2020). ARGONAUTE1 and ARGONAUTE4 regulate gene expression and hypoxia tolerance. *Plant Physiol.*, 182(1), 287–300.
- Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F., & Richmond, T. J., (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, 389(6648), 251–260.
- Luo, M., Cheng, K., Xu, Y., Yang, S., & Wu, K., (2017). Plant responses to abiotic stress regulated by histone deacetylases. *Front. Plant Sci.*, 8, 2147.
- Luo, M., Liu, X., Singh, P., Cui, Y., Zimmerli, L., & Wu, K., (2012). Chromatin modifications and remodeling in plant abiotic stress responses. *Biochim. Biophys. Acta*, 1819(2), 129–136.
- Luo, M., Wang, Y. Y., Liu, X., Yang, S., Lu, Q., Cui, Y., & Wu, K., (2012). HD2C interacts with HDA6 and is involved in ABA and salt stress response in *Arabidopsis*. *J. Exp. Bot.*, 63(8), 3297–3306.
- Luo, X., & He, Y., (2020). Experiencing winter for spring flowering: A molecular epigenetic perspective on vernalization. *J. Integr. Plant Biol.*, 62(1), 104–117.
- Lv, D. K., Bai, X., Li, Y., Ding, X. D., Ge, Y., Cai, H., Ji, W., Wu, N., & Zhu, Y. M., (2010). Profiling of cold-stress-responsive miRNAs in rice by microarrays. *Gene*, 459(1, 2), 39–47.
- Marfella, C. G., & Imbalzano, A. N., (2007). The Chd family of chromatin remodelers. *Mutat Res.*, 618(1, 2), 30–40.

- Marmorstein, R., & Zhou, M. M., (2014). Writers and readers of histone acetylation: Structure, mechanism, and inhibition. *Cold Spring Harb. Perspect. Biol.*, 6(7), a018762.
- Marozsán-Tóth, Z., Vashegyi, I., Galiba, G., & Tóth, B., (2015). The cold response of CBF genes in barley is regulated by distinct signaling mechanisms. *J. Plant Physiol.*, 181, 42–49.
- McGinty, R. K., & Tan, S., (2015). Nucleosome structure and function. *Chem Rev.*, 115(6), 2255–2273.
- Min, Y. Z., & Chen, J., (2013). A potential role of microRNAs in plant response to metal toxicity. *Metallomics*, 5(9), 1184–1190.
- Moore, F. C., & Lobell, D. B., (2015). The fingerprint of climate trends on European crop yields. *PNAS.*, 112(9), 2670–2675.
- Mousavi, S. A., Pouya, F. M., Ghaffari, M. R., Mirzaei, M., Ghaffari, A., Alikhani, M., Ghareyazie, M., et al., PlantPREs: A database for plant proteome response to stress. *J. Proteom.*, 143, 69–72.
- Munns, R., & Tester, M., (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59, 651–681.
- Naika, M., Shameer, K., Mathew, O. K., Gowda, R., & Sowdhamini, R., (2013). STIFDB2: An updated version of plant stress-responsive transcription factor database with additional stress signals, stress-responsive transcription factor binding sites and stress-responsive genes in *Arabidopsis* and rice. *Plant Cell Physiol.*, 54(2), e8.
- Nathan, D., Ingvarsdottir, K., Sterner, D. E., Bylebøl, G. R., Dokmanovic, M., Dorsey, J. A., Whelan, K. A., et al., (2006). Histone sumoylation is a negative regulator in *Saccharomyces cerevisiae* and shows dynamic interplay with positive-acting histone modifications. *Genes Dev.*, 20(8), 966–976.
- Neary, J. L., & Carless, M. A., (2020). Methylated DNA immunoprecipitation sequencing (MeDIP-seq): Principles and applications. In: Tollefsbol, T., (ed.), *Epigenetics Methods* (pp. 157–179). Academic Press.
- Nie, W. F., Lei, M., Zhang, M., Tang, K., Huang, H., Zhang, C., Miki, D., et al., (2019). Histone acetylation recruits the SWR1 complex to regulate active DNA demethylation in *Arabidopsis*. *PNAS.*, 116(33), 16641–16650.
- Niederhuth, C. E., & Schmitz, R. J., (2017). Putting DNA methylation in context: From genomes to gene expression in plants. *Biochim. Biophys. Acta Gene Regul. Mech.*, 1860(1), 149–156.
- Noya, I., González-García, S., Bacenetti, J., Fiala, M., & Moreira, M. T., (2018). Environmental impacts of the cultivation-phase associated with agricultural crops for feed production. *J. Clean. Prod.*, 172, 3721–3733.
- Ohama, N., Sato, H., Shinozaki, K., & Yamaguchi-Shinozaki, K., (2017). Transcriptional regulatory network of plant heat stress response. *Trends Plant Sci.*, 22(1), 53–65.
- Onufriev, A. V., & Schiessel, H., (2019). The nucleosome: From structure to function through physics. *Curr. Opin. Struct. Biol.*, 56, 119–130.
- Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R., Church, J. A., et al., (2014). In: Pachauri, R. K., & Meyer, L., (eds.), *Climate Change 2014: Synthesis Report*. Contribution of working groups I, II, and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. IPCC.
- Paixão, J. F. R., Gillet, F. X., Ribeiro, T. P., Bournaud, C., Lourenço-Tessutti, I. T., Noriega, D. D., De Melo, B. P., et al., (2019). Improved drought stress tolerance in *Arabidopsis* by CRISPR/dCas9 fusion with a histone acetyl transferase. *Sci. Rep.*, 9(1), 1–9.

- Pandey, A. K., Gedda, M. R., & Verma, A. K., (2020). Effect of arsenic stress on expression pattern of a rice specific miR156j at various developmental stages and their allied co-expression target networks. *Front. Plant Sci.*, 11, 752.
- Pandey, C., Raghuram, B., Sinha, A. K., & Gupta, M., (2015). miRNA plays a role in the antagonistic effect of selenium on arsenic stress in rice seedlings. *Metallomics*, 7(5), 857–866.
- Pandey, R., MuÈller, A., Napoli, C. A., Selinger, D. A., Pikaard, C. S., Richards, E. J., Bender, J., et al., (2002). Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. *Nucleic Acids Res.*, 30(23), 5036–5055.
- Park, J., Lim, C. J., Shen, M., Park, H. J., Cha, J. Y., Iniesto, E., Rubio, V., et al., (2018). Epigenetic switch from repressive to permissive chromatin in response to cold stress. *PNAS*, 115(23), E5400–E5409.
- Park, M., Keung, A. J., & Khalil, A. S., (2016). The epigenome: The next substrate for engineering. *Genome Biol.*, 17(1), 1–17.
- Park, P. J., (2009). ChIP-seq: Advantages and challenges of a maturing technology. *Nat. Rev. Genet.*, 10(10), 669–680.
- Pavangadkar, K., Thomashow, M. F., & Triezenberg, S. J., (2010). Histone dynamics and roles of histone acetyltransferases during cold-induced gene regulation in *Arabidopsis*. *Plant Mol. Biol.*, 74(1), 183–200.
- Pecinka, A., & Mittelsten, S. O., (2012). Stress-induced chromatin changes: A critical view on their heritability. *Plant Cell Physiol.*, 53(5), 801–808.
- Peng, M., Ying, P., Liu, X., Li, C., Xia, R., Li, J., & Zhao, M., (2017). Genome-wide identification of histone modifiers and their expression patterns during fruit abscission in litchi. *Front. Plant Sci.*, 8, 639.
- Pikaard, C. S., & Scheid, O. M., (2014). Epigenetic regulation in plants. *Cold Spring Harb. Perspect. Biol.*, 6(12), a019315.
- Platt, A., Gugger, P. F., Pellegrini, M., & Sork, V. L., (2015). Genome-wide signature of local adaptation linked to variable C p G methylation in oak populations. *Mol. Ecol.*, 24(15), 3823–3830.
- Popova, O. V., Dinh, H. Q., Aufsatz, W., & Jonak, C., (2013). The RdDM pathway is required for basal heat tolerance in *Arabidopsis*. *Mol. Plant.*, 6(2), 396–410.
- Pourdad, S., & Beg, A., (2003). In Safflower: A suitable oilseed crop for dry-land areas of Iran. *Proc. 7<sup>th</sup> Int. Conference on Development of Drylands* (pp. 32, 33).
- R, M., S. K., Wang, Y., Zhang, X., Cheng, H., Sun, L., He, S., & Hao, F., (2020). Redox components: Key regulators of epigenetic modifications in plants. *Int. J. Mol. Sci.*, 21(4).
- Rahman, M., Lee, S. H., Ji, H. C., Kabir, A. H., Jones, C. S., & Lee, K. W., (2018). Importance of mineral nutrition for mitigating aluminum toxicity in plants on acidic soils: Current status and opportunities. *Int. J. Mol. Sci.*, 19(10), 3073.
- Ramirez-Prado, J. S., Abulfaraj, A. A., Rayapuram, N., Benhamed, M., & Hirt, H., (2018). Plant immunity: From signaling to epigenetic control of defense. *Trends Plant Sci.*, 23(9), 833–844.
- Ransom, M., Dennehey, B. K., & Tyler, J. K., (2010). Chaperoning histones during DNA replication and repair. *Cell*, 140(2), 183–195.
- Rascio, N., & Navari-Izzo, F., (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Sci.*, 180(2), 169–181.

- Ravichandran, S., Ragupathy, R., Edwards, T., Domaratzki, M., & Cloutier, S., (2019). MicroRNA-guided regulation of heat stress response in wheat. *BMC Genomics*, 20(1), 1–16.
- Ren, B., Robert, F., Wyrick, J. J., Aparicio, O., Jennings, E. G., Simon, I., Zeitlinger, J., et al., (2000). Genome-wide location and function of DNA binding proteins. *Science*, 290(5500), 2306–2309.
- Robertson, A. L., & Wolf, D. E., (2012). The role of epigenetics in plant adaptation. *Trends Ecol. Evol.*, 4(1), e4.
- Rozema, J., & Flowers, T., (2008). Crops for a salinized world. *Science*, 1478–1480.
- Sadakierska-Chudy, A., & Filip, M., (2015). A comprehensive view of the epigenetic landscape. Part II: Histone post-translational modification, nucleosome level, and chromatin regulation by ncRNAs. *Neurotox Res.*, 27(2), 172–197.
- Sahu, P. P., Pandey, G., Sharma, N., Puranik, S., Muthamilarasan, M., & Prasad, M., (2013). Epigenetic mechanisms of plant stress responses and adaptation. *Plant Cell Rep.*, 32(8), 1151–1159.
- Samac, D. A., & Tesfaye, M., (2003). Plant improvement for tolerance to aluminum in acid soils—a review. *Plant Cell, Tissue Organ Cult.*, 75(3), 189–207.
- Sanchez, D. H., & Paszkowski, J., (2014). Heat-induced release of epigenetic silencing reveals the concealed role of an imprinted plant gene. *PLoS Genet.*, 10(11), e1004806.
- Sani, E., Herzyk, P., Perrella, G., Colot, V., & Amtmann, A., (2013). Hyperosmotic priming of *Arabidopsis* seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome. *Genome Biol.*, 14(6), 1–24.
- Sasidharan, R., Bailey-Serres, J., Ashikari, M., Atwell, B. J., Colmer, T. D., Fagerstedt, K., Fukao, T., et al., (2017). Community recommendations on terminology and procedures used in flooding and low oxygen stress research. *New Phytol.*, 214(4), 1403–1407.
- Saze, H., & Kakutani, T., (2007). Heritable epigenetic mutation of a transposon-flanked *Arabidopsis* gene due to lack of the chromatin-remodeling factor DDM1. *EMBO J.*, 26(15), 3641–3652.
- Seto, E., & Yoshida, M., (2014). Erasers of histone acetylation: The histone deacetylase enzymes. *Cold Spring Harb Perspect Biol.*, 6(4), a018713.
- Shahid, M., Khalid, S., Abbas, G., Shahid, N., Nadeem, M., Sabir, M., Aslam, M., & Dumat, C., (2015). Heavy metal stress and crop productivity. In: Hakeem, K., (ed.), *Crop Production and Global Environmental Issues* (pp. 1–25). Springer: Cham.
- Shanker, A. K., Maheswari, M., Yadav, S. K., Desai, S., Bhanu, D., Attal, N. B., & Venkateswarlu, B., (2014). Drought stress responses in crops. *Funct. Integr. Genomics*, 14(1), 11–22.
- Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., & Casero, R. A., (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*, 119(7), 941–953.
- Shinozaki, K., & Yamaguchi-Shinozaki, K., (2007). Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.*, 58(2), 221–227.
- Singh, R. K., Jaishankar, J., Muthamilarasan, M., Shweta, S., Dangi, A., & Prasad, M., (2016). Genome-wide analysis of heat shock proteins in C 4 model, foxtail millet identifies potential candidates for crop improvement under abiotic stress. *Sci. Rep.*, 6(1), 1–14.
- Singroha, G., & Sharma, P., (2019). Epigenetic modifications in plants under abiotic stress. In: Meccariello, R., (ed.), *Epigenetics*. IntechOpen.

- Sobhanian, H., Pahlavan, S., & Meyfour, A., (2020). How does proteomics target plant environmental stresses in a semi-arid area? *Mol. Biol. Rep.*, 47(4), 3181–3194.
- Sokol, A., Kwiatkowska, A., Jerzmanowski, A., & Prymakowska-Bosak, M., (2007). Up-regulation of stress-inducible genes in tobacco and *Arabidopsis* cells in response to abiotic stresses and ABA treatment correlates with dynamic changes in histone H3 and H4 modifications. *Planta*, 227(1), 245–254.
- Sridhar, V. V., Kapoor, A., Zhang, K., Zhu, J., Zhou, T., Hasegawa, P. M., Bressan, R. A., & Zhu, J. K., (2007). Control of DNA methylation and heterochromatic silencing by histone H2B deubiquitination. *Nature*, 447(7145), 735–738.
- Srivastava, S., Suprasanna, P., D'souza, S., (2012). Mechanisms of arsenic tolerance and detoxification in plants and their application in transgenic technology: A critical appraisal. *Int. J. Phytoremediation*, 14(5), 506–517.
- Sudan, J., Raina, M., & Singh, R., (2018). Plant epigenetic mechanisms: Role in abiotic stress and their generational heritability. *3 Biotech*, 8(3), 172.
- Sudarsanam, P., & Winston, F., (2000). The Swi/Snf family nucleosome-remodeling complexes and transcriptional control. *Trends Genet.*, 16(8), 345–351.
- Sun, F., Guo, W., Du, J., Ni, Z., Sun, Q., & Yao, Y., (2013). Widespread, abundant, and diverse TE-associated siRNAs in developing wheat grain. *Gene*, 522(1), 1–7.
- Sunkar, R., & Zhu, J. K., (2004). Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. *Plant Cell*, 16(8), 2001–2019.
- Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R., (2014). Abiotic and biotic stress combinations. *New Phytol.*, 203(1), 32–43.
- Tack, J., Barkley, A., & Nalley, L. L., (2015). Effect of warming temperatures on US wheat yields. *PNAS*, 112(22), 6931–6936.
- Tan, J., He, S., Yan, S., Li, Y., Li, H., Zhang, H., Zhao, L., & Li, L., (2014). Exogenous EDDS modifies copper-induced various toxic responses in rice. *Protoplasma*, 251(5), 1213–1221.
- Tang, L., Nogales, E., & Ciferri, C., (2010). Structure and function of SWI/SNF chromatin remodeling complexes and mechanistic implications for transcription. *Prog. Biophys. Mol. Biol.*, 102(2, 3), 122–128.
- Taverna, S. D., Li, H., Ruthenburg, A. J., Allis, C. D., & Patel, D. J., (2007). How chromatin-binding modules interpret histone modifications: Lessons from professional pocket pickers. *Nat. Struct. Biol.*, 14(11), 1025–1040.
- Teakle, N., Real, D., & Colmer, T., (2006). Growth and ion relations in response to combined salinity and waterlogging in the perennial forage legumes *Lotus corniculatus* and *Lotus tenuis*. *Plant Soil*, 289(1), 369–383.
- Teperino, R., Schoonjans, K., & Auwerx, J., (2010). Histone methyl transferases and demethylases; can they link metabolism and transcription? *Cell Metab.*, 12(4), 321–327.
- Tricker, P. J., (2015). Transgenerational inheritance or resetting of stress-induced epigenetic modifications: Two sides of the same coin. *Front. Plant Sci.*, 6, 699.
- Tricker, P. J., Gibbings, J. G., Rodríguez, L. C. M., Hadley, P., & Wilkinson, M. J., (2012). Low relative humidity triggers RNA-directed de novo DNA methylation and suppression of genes controlling stomatal development. *J. Exp. Bot.*, 63(10), 3799–3813.
- Trucchi, E., Mazzarella, A. B., Gilfillan, G. D., Lorenzo, M. T., Schönschetter, P., & Paun, O., (2016). Bs RAD seq: Screening DNA methylation in natural populations of non-model species. *Mol. Ecol.*, 25(8), 1697–1713.

- Tsuji, H., Saika, H., Tsutsumi, N., Hirai, A., & Nakazono, M., (2006). Dynamic and reversible changes in histone H3-Lys4 methylation and H3 acetylation occurring at submergence-inducible genes in rice. *Plant Cell Physiol.*, 47(7), 995–1003.
- Ueda, M., & Seki, M., (2020). Histone modifications form epigenetic regulatory networks to regulate abiotic stress response. *Plant Physiol.*
- Valdés-López, O., Yang, S. S., Aparicio-Fabre, R., Graham, P. H., Reyes, J. L., Vance, C. P., & Hernández, G., (2010). MicroRNA expression profile in common bean (*Phaseolus vulgaris*) under nutrient deficiency stresses and manganese toxicity. *New Phytol.*, 187(3), 805–818.
- Van, G. T. P., Wagemaker, N. C., Wouters, B., Vergeer, P., Ouborg, J. N., & Verhoeven, K. J., (2016). epiGBS: Reference-free reduced representation bisulfite sequencing. *Nat. Methods*, 13(4), 322–324.
- Vaniushin, B., & Belozerskii, A., (1959). *The Nucleotide Composition of Deoxyribonucleic Acid in Higher Plants* (Vol. 129, No. 4, pp. 944–947). Reports of the Academy of Sciences of the USSR.
- Vanyushin, B., (2014). Epigenetics today and tomorrow. *Russ. J. Genet. Appl. Res.*, 4(3), 168–188.
- Vanyushin, B., Kadyrova, D. K., Karimov, K., & Belozerskii, A., (1971). Minor bases in DNA of higher plants. *Biochemistry*.
- Verhoeven, K. J., Jansen, J. J., Van, D. P. J., & Biere, A., (2010). Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol.*, 185(4), 1108–1118.
- Volkova, P. Y., Geras' Kin, S., Horemans, N., Makarenko, E., Saenen, E., Duarte, G., Nauts, R., et al., (2018). Chronic radiation exposure as an ecological factor: Hypermethylation and genetic differentiation in irradiated Scots pine populations. *Environ. Pollut.*, 232, 105–112.
- Vyse, K., Faivre, L., Romich, M., Pagter, M., Schubert, D., Hinch, D. K., & Zuther, E., (2020). Transcriptional and post-transcriptional regulation and transcriptional memory of chromatin regulators in response to low temperature. *Front. Plant Sci.*, 11, 39.
- Wang, B., Sun, Y., Song, N., Wang, X., Feng, H., Huang, L., & Kang, Z., (2013). Identification of UV-B-induced microRNAs in wheat. *Genet Mol. Res.*, 12(4), 4213–4221.
- Wang, J., Meng, X., Dobrovolskaya, O. B., Orlov, Y. L., & Chen, M., (2017). Non-coding RNAs and their roles in stress response in plants. *Genomics, Proteomics & Bioinformatics*, 15(5), 301–312.
- Wang, J., Meng, X., Yuan, C., Harrison, A. P., & Chen, M., (2016). The roles of cross-talk epigenetic patterns in *Arabidopsis thaliana*. *Brief Funct. Genomics.*, 15(4), 278–287.
- Wang, Z., Wang, F., Tang, J., Huang, Z., Xiong, A., & Hou, X., (2014). C-repeat binding factor gene family identified in non-heading Chinese cabbage is functional in abiotic and biotic stress response but different from that in *Arabidopsis*. *Acta Physiol. Plant.*, 36(12), 3217–3229.
- Waterborg, J. H., (2002). Dynamics of histone acetylation *in vivo*. A function for acetylation turnover? *Biochem. Cell Biol.*, 80(3), 363–378.
- Weinhold, B., (2006). Epigenetics: The science of change. *Environ Health Perspect.*, 114(3), A160–A167.
- Xiao, J., Lee, U. S., & Wagner, D., (2016). Tug of war: Adding and removing histone lysine methylation in *Arabidopsis*. *Curr. Opin. Plant Biol.*, 34, 41–53.

- Xu, R., Wang, Y., Zheng, H., Lu, W., Wu, C., Huang, J., Yan, K., et al., (2015). Salt-induced transcription factor MYB74 is regulated by the RNA-directed DNA methylation pathway in *Arabidopsis*. *J. Exp. Bot.*, 66(19), 5997–6008.
- Xu, Y., Zhang, S., Lin, S., Guo, Y., Deng, W., Zhang, Y., & Xue, Y., (2017). WERAM: A database of writers, erasers and readers of histone acetylation and methylation in eukaryotes. *Nucleic Acids Res.*, 45(D1), D264–D270.
- Yang, C., Shen, W., Chen, H., Chu, L., Xu, Y., Zhou, X., Liu, C., et al., (2018). Characterization and subcellular localization of histone deacetylases and their roles in response to abiotic stresses in soybean. *BMC Plant Biol.*, 18(1), 1–13.
- Yang, R., Hong, Y., Ren, Z., Tang, K., Zhang, H., Zhu, J. K., & Zhao, C., (2019). A role for PICKLE in the regulation of cold and salt stress tolerance in *Arabidopsis*. *Front. Plant Sci.*, 10, 900.
- Yin, X., Romero-Campero, F. J., De Los, R. P., Yan, P., Yang, J., Tian, G., Yang, X., et al., (2021). H2AK121ub in *Arabidopsis* associates with a less accessible chromatin state at transcriptional regulation hotspots. *Nat. Commun.*, 12(1), 1–12.
- Yolcu, S., Ozdemir, F., Güler, A., & Bor, M., (2016). Histone acetylation influences the transcriptional activation of POX in *Beta vulgaris* L. and *Beta maritima* L. under salt stress. *Plant Physiol. Biochem.*, 100, 37–46.
- Yu, Z., Wang, X., Mu, X., & Zhang, L., (2019). RNAi mediated silencing of dehydrin gene WZY2 confers osmotic stress intolerance in transgenic wheat. *Funct. Plant Biol.*, 46(10), 877–884.
- Zemach, A., McDaniel, I. E., Silva, P., & Zilberman, D., (2010). Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science*, 328(5980), 916–919.
- Zhang, C. J., Hou, X. M., Tan, L. M., Shao, C. R., Huang, H. W., Li, Y. Q., Li, L., et al., (2016). The *Arabidopsis* acetylated histone-binding protein BRAT1 forms a complex with BRP1 and prevents transcriptional silencing. *Nat. Commun.*, 7(1), 1–13.
- Zhang, H., Lang, Z., & Zhu, J. K., (2018). Dynamics and function of DNA methylation in plants. *Nat. Rev. Mol. Cell Biol.*, 19(8), 489–506.
- Zhang, S., Yue, Y., Sheng, L., Wu, Y., Fan, G., Li, A., Hu, X., ShangGuan, M., & Wei, C., (2013). PASmiR: A literature-curated database for miRNA molecular regulation in plant response to abiotic stress. *BMC Plant Biol.*, 13(1), 1–8.
- Zhang, X., Fowler, S. G., Cheng, H., Lou, Y., Rhee, S. Y., Stockinger, E. J., & Thomashow, M. F., (2004). Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant *Arabidopsis*. *Plant J.*, 39(6), 905–919.
- Zhang, X., Li, C., Tie, D., Quan, J., Yue, M., & Liu, X., (2021). Epigenetic memory and growth responses of the clonal plant *Glechoma longituba* to parental recurrent UV-B stress. *Funct. Plant Biol.*, 48(8), 827–838.
- Zhang, X., Yazaki, J., Sundaresan, A., Cokus, S., Chan, S. W. L., Chen, H., Henderson, I. R., et al., (2006). Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. *Cell*, 126(6), 1189–1201.
- Zhao, J., Lu, Z., Wang, L., & Jin, B., (2021). Plant responses to heat stress: Physiology, transcription, noncoding RNAs, and epigenetics. *Int. J. Mol. Sci.*, 22(1), 117.
- Zhao, S., Zhang, B., Yang, M., Zhu, J., & Li, H., (2018). Systematic profiling of histone readers in *Arabidopsis thaliana*. *Cell Rep.*, 22(4), 1090–1102.
- Zhao, T., Zhan, Z., & Jiang, D., (2019). Histone modifications and their regulatory roles in plant development and environmental memory. *J. Genet. Genomics*, 46(10), 467–476.



- Zheng, B., & Chen, X., (2011). Dynamics of histone H3 lysine 27 trimethylation in plant development. *Curr. Opin. Plant Biol.*, 14(2), 123–129.
- Zheng, S. J., (2010). Crop production on acidic soils: Overcoming aluminium toxicity and phosphorus deficiency. *Ann. Bot.*, 106(1), 183, 184.
- Zheng, X., Chen, L., Xia, H., Wei, H., Lou, Q., Li, M., Li, T., & Luo, L., (2017). Transgenerational epimutations induced by multi-generation drought imposition mediate rice plant's adaptation to drought condition. *Sci. Rep.*, 7(1), 1–13.
- Zheng, Y., Thomas, P. M., & Kelleher, N. L., (2013). Measurement of acetylation turnover at distinct lysines in human histones identifies long-lived acetylation sites. *Nat. Commun.*, 4, 2203.
- Zhou, S., Chen, Q., Sun, Y., & Li, Y., (2017). Histone H2B monoubiquitination regulates salt stress-induced microtubule depolymerization in *Arabidopsis*. *Plant Cell Environ.*, 40(8), 1512–1530.
- Zhou, Y., Romero-Campero, F. J., Gómez-Zambrano, Á., Turck, F., & Calonje, M., (2017). H2A monoubiquitination in *Arabidopsis thaliana* is generally independent of LHP1 and PRC2 activity. *Genome Biol.*, 18(1), 1–13.
- Zhou, Z. S., Zeng, H. Q., Liu, Z. P., & Yang, Z. M., (2012). Genome-wide identification of *Medicago truncatula* microRNAs and their targets reveals their differential regulation by heavy metal. *Plant Cell Environ.*, 35(1), 86–99.
- Zhu, J. K., (2016). Abiotic stress signaling and responses in plants. *Cell*, 167(2), 313–324.
- Zilberman, D., Gehring, M., Tran, R. K., Ballinger, T., & Henikoff, S., (2007). Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nat. Genet.*, 39(1), 61–69.

## CHAPTER 13

---

# Advances in Proteomics Research in Environmental Stress Response in Plants

KASINATHAN RAKKAMMAL, P. S. JEEVAN RAM, and  
MANIKANDAN RAMESH\*

*Department of Biotechnology, Science Campus, Alagappa University,  
Karaikudi – 630003, Tamil Nadu, India*

*\*Corresponding author. E-mail: mrbiotech.alu@gmail.com*

---

### ABSTRACT

Proteomics is one of the most advanced molecular techniques for investigating protein profiles in stressed plants. Plants respond to stress by modifying their protein, either by up or downregulating their existing protein pool or by synthesizing new proteins. The majority of the proteins were associated with the antioxidative defense mechanism of the plants. In recent years, proteomics has provided scientists with the tremendous ability to examine protein profiles in stressed plants as it correlates the probable link between protein abundance and plant stress tolerance. To get a better knowledge of abiotic stress sensing and intracellular stress signal transduction pathways in subcellular organelles could provide much more detailed information about the fundamental mechanism of stress responses. In this chapter, we reviewed all major contributions related to improved protein extraction protocols, abiotic stress inducers, and their sequels on organelle proteomes to understand the protein level involvement of abiotic stress tolerance mechanism in plants.

### 13.1 INTRODUCTION

Any environmental condition that harms the growth and development of plants, crop quality, and yield is referred to as stress. The stress response is induced in all plants, resulting in either stress escape (survive under stress treatment in metabolically inactive dormant phase) or stress resistance. Stress resistance comprises stress avoidance, or a plant response aimed at maintaining unstressed conditions at the cellular and tissue levels, as well as stress tolerance, or an active plant stress response to a changing environment (Kosava et al., 2018).

Proteins are important in stress response because they are directly involved in developing new phenotypes by adapting physiological characteristics to environmental changes. Proteins are the crucial executors of cellular processes and key players in the maintenance of cellular homeostasis; proteins contribute directly to the formation of new plant phenotypes by regulating physiological properties to adapt to changes in the environment (Rodziewicz et al., 2014; Liu et al., 2019). However, the individual protein behavior rarely reflects the plant's complex network of signals and dynamic regulation of cellular processes in response to abiotic stress. In addition, it is considered that the plant system reacts to abiotic stress as a complex system with numerous signal connections and crosstalk, as well as a diverse variety of stress tolerance-related proteins. As a result, several proteins in the stress response are likely to be working together and play a crucial role in the tolerance mechanism. Many of our previous research of plant responses to abiotic stress emerges from genetic, genomic, and transcriptomic strategies. Although the study of gene and mRNA abundance has considerably aided our understanding of the plant response to various stress, there is usually a weak connection between mRNA expression levels and protein levels (Liu et al., 2019). Proteomic research is conducted to discover novel proteins, as well as disclose their activities and the regulatory networks that control their expression (Hakeem et al., 2012). Proteomics is growing rapidly in three areas of plant science: (i) cellular and subcellular; (ii) structural and developmental biology; and (iii) physiological and genetic research.

Recent advances in quantitative proteomics studies utilizing high-resolution and mass-accuracy instruments significantly contributed to the identification of proteins and their expression profile under stressed and normal conditions (Ghosh & Xu, 2014; Ahmad et al., 2016). Proteomic analysis for both model and non-model plant species was achieved through systematic high-throughput methodologies including 2D-PAGE (2 dimensional-poly

acrylamide gel-electrophoresis), MALDI-TOF/MS/MS, and LC-MS/MS, as well as elucidation of protein functions and protein functional networks in plant metabolic and signaling pathways, is accomplished using protein mapping, characterization of PTMs and bioinformatic approaches and databases for protein-protein interaction (Holman et al., 2013; Ahmad et al., 2016). In this chapter, we discussed the impact of proteomic analysis on the understanding of the accumulation of stress-related protein along with their biological function and cellular localization.

### **13.2 PROTEIN CELLULAR FRACTIONATION AND PURIFICATION METHODS**

Plant cells are made up of a cell wall (ECM: extracellular matrix) is a component of the symplast (apoplast and cytosol), which is linked to neighboring cells via plasmodesmata. The plasma membrane separates the apoplast and symplast environments, serving as a dynamic contact between them. Symplast includes double-membrane enveloped organelles (nucleus, plastids, and mitochondria) and single membrane enveloped secretory vesicular compartments (endoplasmic reticulum (ER), Golgi complex, and trans-Golgi network (TGN), vacuoles, peroxisomes, glyoxisomes). Every organelle plays a specific role during plant stress adaptation: the nucleus is the site of stress signal conversion to gene expression, whereas mitochondria and chloroplasts are the localities of aerobic metabolism, which is essential for energy supply during stress acclimation. However, Proteomic research on the organelle response to stress is still in its early stages. Cellular fractionation is an effective strategy for reducing the complexity of cellular proteomes. As a result, in entire proteome extracts, low-abundant organellar proteins are often masked by high-abundant cytosolic proteins.

Centrifugation-based and affinity purification-based approaches are typical applications for subcellular separation and purification. These methods are rather time-consuming, take quite a long time and make it very difficult to get pure fractions. To address the limitations of the preceding approaches, a subcellular separation technique based on several separation principles has been developed (Table 13.1). Newly developed proteomic techniques have revealed a lot of information. This research has enhanced our understanding of the unique proteomic activity of certain organs, which will aid our understanding of how plants acquire abiotic stress tolerance. For example, Laser capture microdissection (LCM) is a potential sampling approach that uses direct microscopic vision and a laser beam to identify

specific cell types from sectioned specimens of heterogeneous tissues (Longuespée et al., 2014). LCM has been successfully applied in rice (Kubo et al., 2013). The FIFFF (Flow Field-Flow Fractionation) method is an elution-based separation method for separating biological macromolecules without focusing on sample components. The ribosome profiles of *Nicotiana benthamiana* were studied using asymmetric FIFFF. It is used to isolate free molecules of complete and subunits of ribosomes under optimal working circumstances (Pitkanen et al., 2014). Another method, FFE (free flow electrophoresis) with two-phase partitioning has been developed to create a population of highly pure plasma membrane vesicles. This high-quality plasma membrane separation approach resulted in a consistent proteomic library of approximately 700 plasma membrane proteins, including peripheral membrane proteins not previously discovered by Michele et al. (2016). Furthermore, chemical proteomics allows for efficient proteome analysis in a natural setting. For intracellular and subcellular proteomic analysis, activity-based protein profiling (ABPP) (Wiedner et al., 2014), engineered ascorbate peroxidase (APEX) (Rhee et al., 2013), organelle-locatable reactive molecules (ORMs) (Yasueda et al., 2016), and proximity-dependent biotin identification (BioID) (Sage et al., 2016) have been developed (Table 13.2).

### 13.3 ORGANELLE PROTEOME

Analyzes of an organelle's proteins are an effective way to figure out how a cell responds under abiotic stress. Although most organelle proteins are nuclear-encoded, certain organelles, such as chloroplasts and mitochondria, have unique genetic material that facilitates them to produce proteins on their own (Agrawal et al., 2011). The interconnections between organelles in plant cells are influenced by abiotic stress, which has an impact on protein regulation and secretion in cellular organelles and compartments. Several secretory mechanisms implicated in plant cell protein targeting have been described in response to abiotic stress (Mitoma & Ito, 1992; Furman et al., 2003). To investigate the organelle proteins involved in the stress response, particularly those with regulatory or protein targeting functions would significantly improve our understanding of the cellular stress response (Hossain et al., 2012). A summary of the key proteins is provided, with changes in relative abundance under stress in the various compartments (Figure 13.1). More detailed information on proteomic experiments focused on subcellular proteomics in abiotic stress-treated plants is provided in Table 13.1, including the mitochondria, nucleus, chloroplasts, cell wall, and plasma membrane.

**TABLE 13.1** Techniques Used in Plant Proteomic Analyzes for Subcellular Separation

Plant Species	Subcellular Organelles	Organ	Methods	References
<i>Hordeum vulgare</i>	Nucleus	Root	Sorting via flow cytometry with a moderate formaldehyde fixation	Petrovska et al. (2014)
<i>Nicotiana benthamiana</i>	Ribosome	Transgenic lines	Asymmetric flow field-flow fractionation	Pitkanen et al. (2014)
<i>Medicago sativa</i>	Cell wall proteins	Stems	CaCl <sub>2</sub> , EGTA, and LiCl-complemented buffers were used in the extraction process	Printz et al. (2015)
<i>Solanum tuberosum</i>	Mitochondria	Tuber	Centrifuge and layered in a Percoll density gradient of 50%, 28%, and 20% in mannitol buffer	Salvato et al. (2014)
<i>Arabidopsis thaliana</i>		Leaf	Centrifugation combined with 1D-Blue native (BN) PAGE	Senkler et al. (2017)
<i>Arabidopsis thaliana</i>	Plasma membrane	Seedlings	Two-phase partitioning combined with free-flow electrophoresis	Michele et al. (2016)
<i>Malus domestica</i>	Chloroplasts	<i>In vitro</i> shoots	Sorbitol-based isolation medium with blender and centrifugation	Morkunaite-Haimi et al. (2018)

**TABLE 13.2** Organellar Proteomics Studies in Plants Response to Abiotic Stress

Plant Species	Organ Used for Fractionation	Experimental Condition	Technique Used	No. of Identified Proteins	DAP (Differentially Abundant Proteins)	References
Nucleus						
<i>Arabidopsis thaliana</i>	Leaves	Cold	2D-MALDI-TOF MS	184	Up: TFs: AtMYB2, MYB34, MYC, bZIP, OBF4, bHLH; DNA and RNA-associated proteins: helicase C in DEAD/DEAH box, U2 sn ribonucleoprotein A, CHP-rich Zn-finger protein; DNA-damage-repair protein DRT102; chaperones: HSP70, HSP90, Hsc70-1; ribosomal proteins: 60S acidic ribosomal proteins P0, P2-A, L12  Down: GLP, calmodulin, 20S proteasome $\alpha$ , HSF8	Bae et al. (2003)
<i>Cicer arietinum</i>	Seedlings	–	2-DE/LC-TOF MS	147	Nucleocytoplasmic transport proteins – Ran protein, RanBP: Ran-binding protein; cell signaling proteins: FCA protein, 14-3-3 protein, protein kinase, serine-threonine kinase, histidine kinase, receptor-like protein kinase.  Chromatin remodeling: HDAC: histone deacetylase, Zmet3: DNA cytosine methyltransferase, MFP1: MAR binding filament-like protein 1, H3, H2B, actin, kinetin.  Molecular chaperones: dnaK, dnaJ, Hsp70, sHsps; ROS pathway related proteins: NADPH oxidase, SOD, APX, GPX, glyoxalase	Pandey et al. (2008)
<i>Oryza sativa</i>	Seedlings	Drought	2-DE/LC-ESI-MS/MS	109	Up: Cu/Zn-SOD, 2-Cys Prx, PR10a, GLP, chitinase, endo-1,3-glucanase; ribosomal proteins: Up: 50S L-22, S6: Down: L12, 60S P2A	Choudhary et al. (2009)

**TABLE 13.2** (Continued)

Plant Species	Organ Used for Fractionation	Experimental Condition	Technique Used	No. of Identified Proteins	DAP (Differentially Abundant Proteins)	References
<i>Xerophyta viscosa</i>	Leaves	Drought	2D-MALDI-TOF MS/MS	438	Up: Zn-finger helicase TF; gene regulation: non-LTR retrotransposons, maturases; chaperonin; EF-Tu precursor, ribosomal L28	Abdalla et al. (2010)
<i>Cicer arietinum</i>	Seedlings	Drought	2DE ESI-MS/MS	75	Genotypic differences: H2B, H3 – up in T, down in S; DNA cytosine methyltransferase Zmet3 – up in T; ROS scavenging – APX, SOD, GPX – up in T	Subba et al. (2013)
<i>Glycine max</i>	Root tip	Flooding	nano-LC MS/MS.	39	Down: epsilon2-COP, RACK1, beta-catenin, splicing factor PWI domain-containing, clathrin heavy chain	Komatsu et al. (2014)
<i>Glycine max</i> phosphoproteome	Root tip	Flooding	LC-MS/MS	14	Zinc finger protein, glycine rich protein, and Rrp5	Yin & Komatsu (2015)
<i>Glycine max</i>	Root tip	Flooding	–	365	Down: H1, H3; NOP1/NOP56; 60S preribosome; pre-mRNA processing (spliceosome components)	Yin & Komatsu (2016)
Mitochondria <i>Pisum sativum</i>	Leaves	Drought, Cold Herbicide (Paraquat – 662.5 mg/L)	2DE Q-TOF MS; BN-PAGE	29	Up: MDH, GDC, SHMT isoforms; ATP synthase $\alpha$ , $\beta$ , $\gamma$ ; HSP22, HSP70, HSP90, chaperonin 10; $\beta$ CA	Taylor et al. (2005)



**TABLE 13.2** (Continued)

Plant Species	Organ Used for Fractionation	Experimental Condition	Technique Used	No. of Identified Proteins	DAP (Differentially Abundant Proteins)	References
<i>Oryza sativa</i>	Root tip	Salt	2DE-IEF/ SDS-PAGE	8	Up: Glycoside hydrolase, Cu/Zn-SOD, 20S proteasome subunit, HSP70.  Down: cytochrome-c oxidase subunit 6b, ATP synthase beta subunit S-adenosylmethionine synthetase 2, transcription initiation factor eIF-3 epsilon	Chen et al. (2009)
<i>Triticum aestivum</i> Wyalkatchem (T), Calingiri, Janz (S)	Shoots	200 mM NaCl	Isolation: PVP gradient; acetone extraction; 2D-DIGE LC-MS/MS	192	Up: Mn-SOD, AOX, VDAC  Down: CS, NDPK, outer mitochondrial membrane porin  Genotypic differences: AOX, Mn-SOD – increased in T	Jacoby et al. (2010)
<i>Glycine max</i>	Root and hypocotyl	Flooding	2-DE/BN-PAGE/MALDI TOF MS	52	Up: NADH-ubiquinone oxidoreductase, TCA cycle enzymes; GroES chaperonin, EF-Tu, porin, VDAC.  Down: cytochrome c, Tim, Tom20; protein components of complexes III, IV, and V of ETC	Komatsu et al. (2011)
<i>Pisum sativum</i>	Leaves	150 mM NaCl	2-DE/MALDI TOF MS	–	SNO modified proteins: ATP synthase $\beta$ , HSP90, SHMT; Prx IIF activity were decreased	Camejo et al. (2013)
<i>Triticum aestivum</i> Chinese Spring (S), <i>T. aestivum</i> $\times$ <i>Lophopyrum elongatum</i> amphiploid (T)	Shoot and root	200 mM NaCl	100% acetone (leaf), TCA/acetone (root); 2D-DIGE MALDI-TOF/ TOF; HPLC Q-TOF MS/MS	68	Genotypic differences: Mn-SOD, MDH, aconitase, SHMT, $\beta$ -CAS – increased in T; Up in shoot-aspartate aminotransferase, GDH	Jacoby et al. (2013)

**TABLE 13.2** (Continued)

Plant Species	Organ Used for Fractionation	Experimental Condition	Technique Used	No. of Identified Proteins	DAP (Differentially Abundant Proteins)	References
Chloroplasts						
<i>Arabidopsis thaliana</i>	Leaves	Cold	2D-DIGE	43	Up: AOC2; ATP synthase $\alpha$ , $\beta$ , $\gamma$ , $\delta$ , SHMT; FIB; CAT-2, glyoxalase I, Trx m, PGM.  Down: RubisCO activase (RCA), 2-Cys PrxA,B; CA-1; PGK, PRK, quinone oxidoreductase	Goulas et al. (2006)
<i>Zea mays</i>	Leaves	1, 25 mM NaCl	2DE-MALDI-TOF	12	Up: ferredoxin-NADP reductase, 23 kDa PSII, FtsH-like; ATP synthase CF1 $\epsilon$ .  Down: ATP synthase CF1 $\alpha$ , LHC <i>a/b</i> binding protein	Zorb et al. (2009)
<i>Glycine max</i>	Leaves	Ozone stress	2DE-MALDI-TOF/MS	32	Down: RuBisCO activase, RuBisCO large and small subunits, sedoheptulose-1,7-bisphosphatase, chlorophyll <i>a/b</i> -binding protein, ferredoxin-NADP reductase, Cytochrome <i>c</i> -553-like, Cytochrome <i>b6-f</i> complex	Ahsan et al. (2010)
<i>Populus tremula</i>	Leaves	Ozone stress	2-DE/MALDI TOF MS	27	Down: ferredoxin-NADP <sup>+</sup> oxidoreductase (FNR), ATPase $\beta$ subunit, RuBisCO activase, RuBisCO large subunit, APX	Bohler et al. (2011)
<i>Triticum aestivum</i>	Leaves	150 mM NaCl	2-DE/LTQ-FTICR	65	Up: Rubisco, glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase, photosystem I, and pyridoxal biosynthesis protein PDX1.2 and PDX1.3.  Down: ATP synthase $\alpha$ , $\beta$ , $\gamma$ , V-type proton ATPase subunits	Kamal et al. (2012)

**TABLE 13.2** (Continued)

Plant Species	Organ Used for Fractionation	Experimental Condition	Technique Used	No. of Identified Proteins	DAP (Differentially Abundant Proteins)	References
<i>Festuca arundinacea</i> – (T), (S)	Leaves	Drought	2-DE; nano-UPLC MS/MS	82	Up: RubisCO activase. Down: lipocalin  Genotypic difference: OEE2, lipocalin – up in T down in S	Kosmala et al. (2012)
<i>Solanum lycopersicum</i>	Leaves	Drought and recovery	2D-DIGE nanoLC-ESI-LIT-MS/MS	57	Photosynthesis (PC, chl binding protein4; PGK, PRK), ATP synthase CF1 $\beta$ , $\gamma$ , APX1, PAP	Tamburino et al. (2017)
Cell Wall						
<i>Zea mays</i>	Primary root elongation zone	Drought	2DE HPLC-ESI-Q-TOF MS	152	Cell wall apoplast proteins: expansion, XET, $\beta$ -D-glucosidase	Zhu et al. (2007)
<i>Cicer arietinum</i>	Leaves	Drought	2-DE/MALDI TOF/MS	134	APX, Trx m, mannose lectin, glyoxalase I, GRPs, NDPK, receptor-like kinase CHRK1, protein kinase 2; cell wall modification: cellulose synthase-like, glucan <i>endo</i> -1,3- $\beta$ -D-glucosidase; Met synthase	Bhushan et al. (2007)
<i>Glycine max</i>	Root and hypocotyl	Waterlogging	2-DE/MALDI TOF/MS	204	Down: GLP, LOX, stem glycoprotein, Cu/Zn-SOD; pI change in copper amino oxidase	Komatsu et al. (2010)
<i>Oryza sativa</i>	Leaves	Drought	2-DE/MALDI TOF/MS	192	Cell signaling proteins: 14-3-3; inositol phosphatase; GF14-b, GF14-c; 20S proteasome $\alpha$ ; oligopeptidase A-like; ALDO, ENO, PRK; SAM 2-demethylmenaquinone methyltransferase-like; ADK, AdoHcyase; Trx h, Trx m, 2-Cys Prx, MDAR; DnaK chaperone	Pandey et al. (2010)

**TABLE 13.2** (Continued)

Plant Species	Organ Used for Fractionation	Experimental Condition	Technique Used	No. of Identified Proteins	DAP (Differentially Abundant Proteins)	References
Plasma Membrane						
<i>Arabidopsis thaliana</i>	Leaves	Cold	2D-MALDI-TOF/MS	38	Phospholipase d- $\delta$ , ribophorin II-like protein, tubulin $\beta$ -2/ $\beta$ -3, elongation factor-1 $\alpha$ (EF-1 $\alpha$ ), hypersensitive-induced reaction (HIR) protein, pollen-specific-like protein	Kawamura & Uemura (2003)
<i>Glycine max</i>	Root and hypocotyl	Flooding	2-DE MS/sequencer, nano-LC-MS/MS	54	Cell signaling proteins: 14-3-3 protein, Ser/Thr protein kinase; Hsc-70; SOD; ATP synthase CF1 $\alpha$	Komatsu et al. (2009)
<i>Glycine max</i>	Seedlings	10% PEG-6,000	2DE, nano-LC-MS/MS	86	Up: calnexin, H <sup>+</sup> -ATPase, ribosomal protein S10, TPI	Nouri & Koamtsu (2010)



### 13.3.1 NUCLEUS

The nucleus is the most critical organelle for controlling protein expression, which is crucial for regulating plant response to abiotic stress. The nuclear protein collaborates along with nucleotide polymers to serve important roles in the nucleus. Furthermore, inside the nucleus, there is a complex network of processes that respond to stress. The discovery of novel nuclear proteins aids our understanding of protein function in providing physiological stress tolerance. There is currently limited information on the proteomic analysis of stress-responsive nuclear protein expression profiles in plants.

The nuclear proteome analysis of model plant *Arabidopsis* response was analyzed under cold stress using 2-DE and MALDI-TOF/MS, respectively. So far, 184 protein spots have been discovered, with 158 of them being involved in a wide range of biological functions. In this, 54 were up-or down-regulated by more than a factor of two. ABA-dependent and ABA-independent transcription factors such as bZIP (basic-region leucine-zipper), bHLH (basic helix-loop-helix), MYB, NAC, and others were also revealed to have known stress response-related motifs or activity (Bae et al., 2003). In addition, expression of the 60S ribosomal protein, small nuclear ribonucleoprotein A' (U2 snRNP-A'), and Hsc70-1 (heat-shock protein chaperon 70-1) was stimulated.

The nuclear proteome of drought-treated chickpea to obtain a good understanding of the molecular mechanisms that represent dehydration-responsive adaptation (Pandey et al., 2008). They identified a total of 205 differentially regulated protein spots, among them 147 differentially expressed proteins involved in gene expression, signal transmission, chaperones, chromatin remodeling, ROS scavenging enzymes, and nucleocytoplasmic transport and Ran-binding protein (RanBP).

To understand a better knowledge of the molecular pathways that represent dehydration-responsive adaptation, a comparable drought-responsive nuclear proteome investigation was carried out in rice (Choudhary et al., 2009). They discovered 109 proteins that are believed to be involved in transcriptional control, chromatin remodeling, signaling, and gene regulation, cell defense and rescue, and protein degradation, among other functions. They also revealed a differential display of nuclear proteome of 150 protein spots whose intensities varied substantially over the dehydration period.

The presence of 27 phosphoproteins controlled by ABA and phosphorylated in response to flooding was discovered in research of nuclear phosphoproteins in soybean root tip during flooding, including zinc-finger/BTB

domain-containing protein 47, glycine-rich protein, and rRNA processing protein (Rrp5) (Yin & Komatsu, 2015). The nuclear fraction of soybean root tip at the initial stage of flooding stress was analyzed by Yin & Komatsu (2016). A total of 365 nuclear proteins were altered in flooding stress. After flooding, the nuclear proteome of soybean revealed different H2A isoforms as well as decreased H1 and H3, indicating extensive chromatin remodeling. Furthermore, in flooded soybean, four exon-junction complex-related proteins including NOP1/NOP56, which operate upstream of 60S pre-ribosome biogenesis, were reduced.

### 13.3.2 MITOCHONDRIA

Mitochondrial proteome study has a new insight into understanding the stress response of plants during abiotic stress factors. Mitochondria is the cell's powerhouse, responsible for nucleotide and vitamin synthesis, lipid, and amino acid metabolism, and the photorespiratory pathway (Miller et al., 2005; Liu et al., 2019). Under stress, the mitochondrial proteome showed an increased level of ROS scavenging enzyme (Mn-SOD) in pea and wheat (Taylor et al., 2005; Jacoby et al., 2010). For a complete examination of mitochondrial activities in plants, an Arabidopsis mitochondrial proteome project was launched. The first 2-DE-PAGE analysis of mitochondrial protein was performed by Kruff et al. (2001). After that, separating proteins for studying the plant mitochondrial proteome under stressful conditions received a lot of interest. They identified 52 protein spots that are collaborating with various processes, such as the citric acid cycle, nucleotide, and amino acid metabolism, respiration, protein biosynthesis, and mitochondrial assembly.

Mitochondria are crucial in cell survival and are a critical location of oxidative stress and cellular response. Common stressors like drought, cold, and herbicides were used to study the reaction of mitochondria in *Pisum sativum* plants. To varying degrees, all of these treatments altered the photosynthetic and respiratory rates of pea leaves, but only herbicides increased lipid peroxidation buildup (Taylor et al., 2005). Mitochondria isolated from stressed pea plants retained their electron transport chain function, but the number of uncoupling proteins, non-phosphorylating respiratory pathways, and oxidative modification of lipoic acid moieties on mitochondrial proteins all changed. The degradation of important matrix enzymes is different under freezing and drought stressors, according to 2D-PAGE and MS analyses of soluble proteins from mitochondria. Furthermore, variable activation of heat

shock proteins and specific losses of other proteins suggest that mitochondria response to various abiotic stressors is diverse at the protein level.

Mitochondrial proteome during salt stress-induced rice programmed cell death (PCD). After 2-DE analysis, eight PCD-related proteins include 4 upregulated (Glycoside hydrolase, mitochondrial heat shock protein 70, 20S proteasome subunit, and Cu/Zn-SOD) and four down-regulated (ATP synthase beta subunit, cytochrome-c oxidase subunit 6b, S-adenosylmethionine synthetase 2, and transcription initiation factor eIF-3 epsilon) proteins were discovered. According to a proteome analysis, ATP synthase may not be the primary ATP generator in rice mitochondria during the early stages of PCD (Chen et al., 2009).

Plant mitochondrial functions have been inhibited by Cd stress was observed in pea (Smiri et al., 2009). During oxidative stress, Arabidopsis mitochondria were investigated to learn more about protein interactions with metal ions and the control of protein activity in plants (Tan et al., 2010). Their findings showed that mitochondrial metal concentration is dynamic and varies under oxidative stress and that various proteins have varied metal affinity and sensitivity to metal ion inactivation. The vulnerability of mitochondrial respiratory chain pathways and matrix enzymes to metal-induced loss of function varied considerably, resulting in selective oxidation events in the mitochondrial proteome.

Under flooding stress, a thorough study of mitochondrial proteins in soybean roots and hypocotyls was carried out. 2-DE and blue native-polyacrylamide gel electrophoresis (BN-PAGE) were used to separate mitochondrial matrix and membrane proteins, respectively (Komatsu et al., 2011). They observed flooding stress increased proteins and metabolites linked to the tricarboxylic acid cycle (TCA) and the  $\gamma$ -amino butyrate shunt, whereas it reduced inner membrane carrier proteins and proteins related to electron transport chain complexes III, IV, and V. The level of NADH and NAD were increased whereas, ATP levels considerably decreased. These findings lead them to the conclusion that flooding stress damages electron transport chains directly, despite the fact that NADH synthesis rises in the mitochondria via the TCA cycle (Komatsu et al., 2011).

The mitochondrial proteome and differences associated with salt tolerance in two contrasting salinity tolerant and sensitive wheat cultivars were studied (Jacoby et al., 2010). They identified 68 unique proteins including Mn-SOD, alternative oxidase (AOX). Cysteine synthase, nucleoside diphosphate kinase, and the voltage-dependent anion channel (VDAC). Both Mn-SOD and AOX are important in scavenging ROS that are produced



in excess under salinity stress. By comparing the reference wheat variety Chinese Spring (CS) to a salt-tolerant amphiploid, the influence of salinity on the mitochondrial protein was studied (AMP). The AMP had a greater abundance of Mn-SOD, MDH (malate dehydrogenase), aconitase, SHMT (serine hydroxymethyltransferase), and  $\beta$ -CAS ( $\beta$ -cyanoalanine synthase) than CS, indicating genotypic variations in mitochondrial composition. In addition, they found organ-specific differences in proteins such as aspartate aminotransferase, GDH (up-regulated in the shoot, down-regulated in root) (Jacoby et al., 2013).

### **13.3.3 CHLOROPLAST**

Abiotic stresses like drought, salt, high or low temperatures, heavy metal cause a significant impact on chloroplasts which is the sites of the photosynthetic machinery. Stress-induced alterations in cellular redox balance, reduction in CO<sub>2</sub> fixation, and NADP<sup>+</sup> regeneration through the Calvin cycle make the photosynthetic apparatus very sensitive. As a result, the photosynthetic electron transport chain becomes too reduced, resulting in the formation of superoxide radicals and singlet oxygen in the chloroplasts. Excess ROS inhibits photosynthesis-related chloroplast protein activities (Hossain et al., 2012; Kosava et al., 2018).

So far, only a few research have been conducted on the abiotic stress responses in chloroplast proteomes. The impact of short-term salt stress on maize chloroplast's proteome was investigated using 2DE MALDI-TOF during the early phases of salt stress (Zorb et al., 2009). This study revealed that ferredoxin-NADP reductase, 23 kDa PSII, FtsH-like; ATP synthase CF1 $\epsilon$  were up-regulated and ATP synthase CF1 $\alpha$ , LHC *a/b* binding proteins were down-regulated.

In a soybean plant, the response to ozone stress and alterations in chloroplast protein expressions were extensively researched (Ahsan et al., 2010). The study found 32 chloroplast proteins that were differentially expressed. Under stress, several proteins involved in photosystem I/II and carbon absorption decreased, possibly explaining why photosynthetic activity dropped in response to ozone. On the other hand, protein responsible for carbon metabolism and antioxidant defense was increased. The authors concluded that sucrose availability may play a crucial role in oxidative stress signaling and antioxidative process regulatory pathways, as well as the

breakdown of starch and increased sucrose levels in response to short-term acute ozone treatment.

Subcellular fractionation and relative protein quantification using the 2D-DIGE method were used to investigate the short and long-term cold stress to chloroplast proteome of *Arabidopsis* (Goulas et al., 2006). Short-term cold stress generated substantial changes in the stromal proteome but little changes in the lumen proteome, whereas cold stress caused minor alterations in the plastid proteomes. Long-term cold stress leads to a modification of both (lumen and stroma) compartments proteomes, with the emergence of novel proteins in the lumen and alterations in protein richness in the stroma. A total of 43 differentially expressed proteins were discovered, including those involved in photosynthesis, phytohormone biosynthesis, stress sensing, signal transduction, and other plastid metabolic functions, all of which are involved in cold stress acclimatization.

The 2D-DIGE method was used to investigate the impact of chloroplast membrane proteins under ozone stress. Extrinsic photosystem proteins along with ATPase subunits were discovered to be varied in number. Under stress conditions, all the protein quantity was decreased except ferredoxin-NADP<sup>+</sup> oxidoreductase. This resulted in a higher level of NADPH, which aids in the detoxification of reactive oxygen species (ROS) created directly or indirectly as a result of oxidative stress (Bohler et al., 2011). The findings suggest that long-term ozone exposure exhausts the cellular defensive mechanisms due to the oxidative character of the stress, resulting in a reduction in the number of photosystem subunits and other chloroplast membrane proteins.

Analysis of leaf chloroplast protein after 3 days of salt treatment in wheat plants showed up-regulation of Rubisco, glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase, photosystem I, and pyridoxal biosynthesis protein PDX1.2 and PDX1.3 and the downregulation of ATP synthase ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) and V-type proton ATPase subunits. These results showed a negative impact of Na<sup>+</sup> on the photosynthetic machinery (Kamal et al., 2012).

Comparative analysis of tolerant and susceptible cultivars of *Festuca arundinacea* was analyzed. Tolerant cultivar showed an up-regulation of RubisCO activase, OEE2 (oxygen-evolving enhancer protein 2), and lipocalin. These proteins were downregulated in the susceptible cultivar (Kosmala et al., 2012). The chloroplast proteome of *Solanum lycopersicum* under drought stress and recovery (Tamburino et al., 2017). They found the downregulation of phosphoribulokinase (PRK), chloroplast sedoheptulose-1,7-bisphosphatase (SBPase), ascorbate peroxidase (Apx-TL29),

transketolase (TK), ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (RbcL), and oxygen-evolving enhancer protein 1 (OEE1).

### **13.3.4 CELL WALL**

The cell wall is a supporting structure as well as an exterior physical barrier that is essential for plant physiology to be stress-free. Cellulose, hemicelluloses, and pectin with polymer-like lignin make up the majority of the plant cell wall (Gall et al., 2015; Voxeur & Hofte, 2016). Because of their critical involvement in stress sensing and signal transduction between the apoplast and symplast, cell wall proteome research has gained a lot of interest in recent years. A comprehensive cell wall protein (CWP) analysis was carried out in the maize root elongation zone for a better understanding of the underlying molecular mechanism of drought stress-responsive adaptation (Zhu et al., 2007). They found 152 water deficit-responsive proteins and categorized them into five main categories relying on their function in the cell wall: ROS metabolism, glucose metabolism, defense, and detoxification, and hydrolases. The findings suggest that stress-induced alterations in CWPs include several mechanisms that are likely to control cell elongation responses. Changes in protein abundance linked to ROS metabolism increased in apo-plastic ROS generation ( $H_2O_2$ , oxalate oxidase, APX, Cu/Zn-SOD, Trx m, germins) in the apical area of the water-stressed root elongation zone. ECM (extracellular matrix) proteins in chickpea seedlings shoot cell walls subjected to drought stress were found to change cell wall modification, cell signaling, metabolism, and cell defense and rescue, harming the molecular mechanism of drought tolerance in plants (Bhushan et al., 2007).

The cell wall proteome of rice shoots under drought stress was examined using 2-DE, which showed 100 proteins that were differentially expressed and may play significant roles in the plant's dehydration tolerance cascade. The stress causes changes in proteins involved in stress signaling (NDPK, involved in  $\gamma$ -phosphate transfers, G-protein signaling, and nucleoside diphosphate kinase), ROS scavenging and detoxification (APX, thioredoxin, glyoxalase I, chitinase), molecular chaperones (DnaK, CPN60, and HSP20), carbohydrate metabolism (phosphoribulokinase, transketolase) and cell wall modifications (enzymes engaged in the phenylpropanoid biosynthesis pathway and methyltransferases involved in the methylation of lignin components) (Pandey et al., 2010). Lower amounts of Cu/Zn-superoxide dismutase (Cu/Zn-SOD), four germin-like proteins, lipoxygenases, and glycoprotein

precursors were found in soybean roots subjected to flooding stress, which is most likely associated with reduced cell wall lignification. The findings reveal that lignification is inhibited by a reduction in reactive oxygen species (ROS) and jasmonate production (Komatsu et al., 2010).

### **13.3.5 PLASMA MEMBRANE**

The plasma membrane is a phospholipid bilayer with embedded proteins that separate the inside of the cell (cytosol) from the outside environment. Membrane proteins make up around half of the total volume of the membrane. One of the key cellular reactions required for the perception of a stress signal and its transmission into the cell, which happens predominantly in the plasma membrane, is a change in gene expression at the protein level (Alexander-son et al., 2004). The identification of potential plasma membrane proteins of *Arabidopsis* leaves related to cold acclimation was done using a mass spectrometric approach. After cold acclimation, a substantial alteration in protein profile was found. MALDI-TOF MS was used to identify a total of 38 proteins. The proteins that are altered in the amount on the first day of cold acclimation are mostly linked with membrane repair, membrane protection against osmotic stress, CO<sub>2</sub> fixation enhancement, and proteolysis (Kawamura & Uemura, 2003).

Plasma membrane proteins were examined using gel-based and gel-free proteomics methods to investigate changes in the soybean subjected to flooding stress reveals flood-induced plasma membrane proteins in soybean. A total of 35 stress-induced new proteins were discovered, the majority of which are involved in the antioxidative defense mechanism of plants. Nouri & Komatsu (2010) investigated the impact of PEG (polyethylene glycol)-induced osmotic stress on the soybean plasma membrane proteome. Plasma membrane purity was confirmed by assessing ATPase activity after purification using a two-phase partitioning technique. Four and eight protein sites were found to be up and down-regulated, respectively, after PEG treatment using gel-based proteomics, whereas 11 and 75 proteins were identified as up- and down-regulated using the nano-LC-MS/MS method. Three isoforms of the H<sup>+</sup>-ATPase, which are involved in ion efflux and show an increase under hyperosmotic stress, have been discovered. According to this result, one protein phosphatase, three isoforms of protein kinases, calnexin, and phototropin are among the proteins that regulate H<sup>+</sup>-ATPase activity.

### **13.4 DEFENSE-RELATED PROTEINS IN PLANT CELL ORGANELLES**

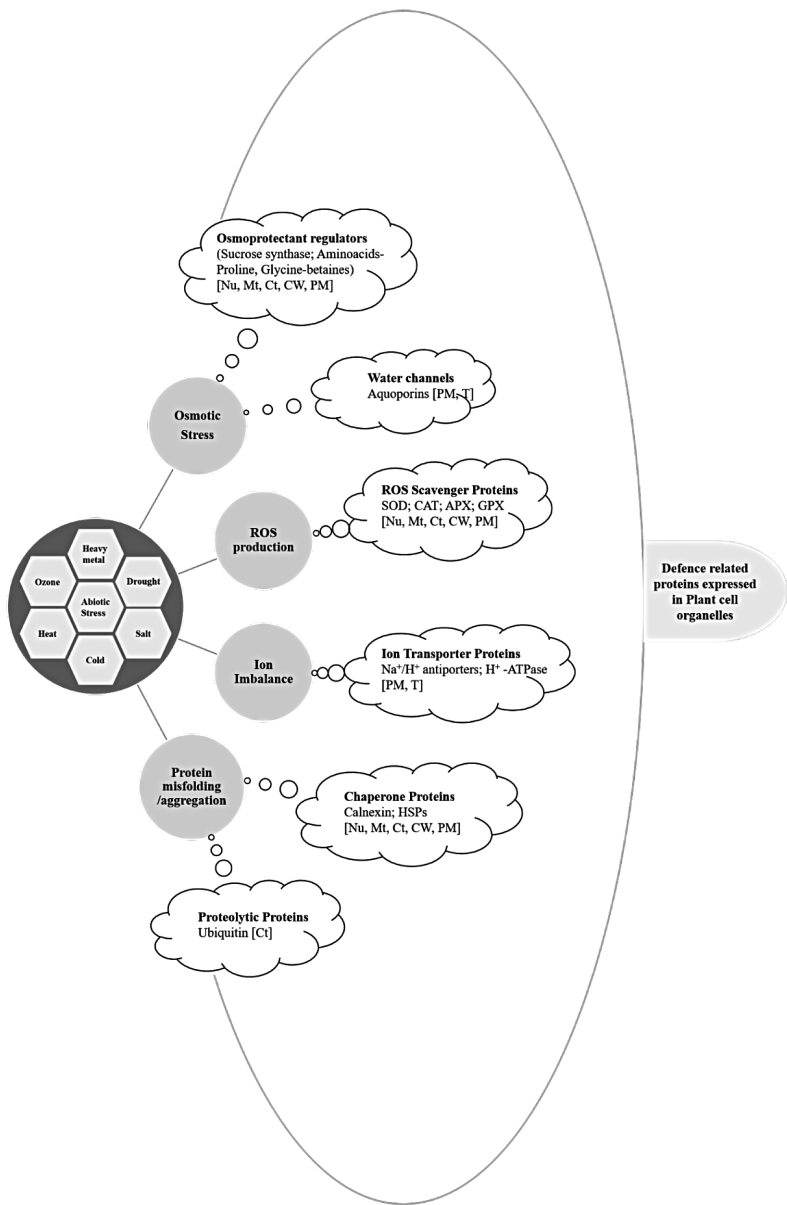
Abiotic stress causes a wide range of alterations in protein expression levels in plant cells. There is no direct relationship between gene expression and the end outcome of protein synthesis and regulation. The presence of a specific protein in a cell does not always imply an increase or reduction in gene expression. Protein expression is governed by several mechanisms, including protein targeting and translocation, as well as post-translational modifications, which are all influenced by stress. Secretory mechanisms and intracellular interactions regulate protein distribution in the cell. When plants are exposed to abiotic stress, they frequently regulate groups of functionally related proteins. Some of these proteins are not involved in the defensive system, but those involved in metabolism, storage, and protein synthesis. According to their roles, defense-related abiotic stress-responsive proteins were classified into six major groups (Lilley et al., 2007; Hossain et al., 2012). These six differentially expressed proteins strongly combat repairing the negative effects of abiotic stress which includes protein misfolding or aggregation, ROS production, ion imbalance, and osmotic potential in plant cell organelles (Figure 13.2).

#### **13.4.1 PROTEOLYTIC PROTEINS**

Proteolysis is a process in which misfolded, unassembled, or mutant proteins were degraded in cells. Among all the organelles ER contains its proteolytic system, which is responsible for protein folding and quality control. Although ubiquitin was generally involved in protein breakdown, proteolysis without ubiquitin is conceivable (Tanaka & Chiba, 1998). The ER degradation machinery's ubiquitin-proteasome system has been discovered to extend into the cytoplasm. The ubiquitin-protein degradation mechanisms have also been discovered in the nucleus (Sommer & Wolf, 1997). Two types of stress-responsive proteins that interact directly with target proteins include proteolytic proteins and molecular chaperones. Proteolysis-related protein activity might be thought of as the cell's final effort to survive in stressful situations. Organelles are designed to work together with other compartments to carry out cellular activities (Hieng et al., 2004).

#### **13.4.2 CHAPERONE PROTEINS**

Chaperone proteins are found in nearly every region of a cell participate in the correct protein folding and assembly of secretory proteins (Cascardo



**FIGURE 13.2** Defense related protein expression in plant organelle to regulate negative effect of abiotic stress. The main locations of the proteins are represented in brackets. (Nu: nucleus; Mt: mitochondria; Ct: chloroplast; CW: cell wall; PM: plasma membrane; T: tonoplast).

et al., 2000). Most of the chaperone proteins are located in the ER, which folds newly synthesized glycoproteins. They contain calreticulin, binding immunoglobulin protein, calnexin, and the majority of heat shock proteins (Williams, 2006). The expression of molecular chaperone proteins helps the cell adapt to adverse environmental conditions by preventing protein aggregation. Various studies have demonstrated that chaperone proteins are regulated differently in response to abiotic stress (Ahsan et al., 2010b). The expression of molecular chaperones in response to stress does not abide by a set of rules. The expression of molecular chaperones is influenced by the plant's age, the consequence, and time of exposure could influence several proteins which would be up or downregulated. Moreover, the expression of molecular chaperones might differ between organelles. As a result, investigations on the molecular chaperone sub-proteome must be carefully designed concerning the stressful circumstances as well as the cells and organelles involved (Wang et al., 2004; Komatsu et al., 2009; Ahsan et al., 2010).

### **13.4.3 ROS SCAVENGER PROTEINS**

Abiotic stress causes cells to produce reactive oxygen species (ROS), such as hydroxyl radicals, hydrogen peroxide ( $H_2O_2$ ), and superoxide anions, which together induce oxidative stress. The stress response of the cell varies depending on the degree of the oxidative stress and can vary from antioxidant defense systems activation to programmed cell death. The major defense against oxidative stress is the creation of ROS scavenger proteins. The predominant scavenging proteins found in plant cells are superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and catalase (CAT) (Blokhina et al., 2003; Apel et al., 2004; Hossain et al., 2009). In organelles like mitochondria and chloroplasts, the production of reactive oxygen species (ROS) is a common biological activity. Stress, on the other hand, induces oxidative damage by increasing the generation of reactive oxygen species (ROS). The presence of scavenger proteins in different plant organelles and subcellular compartments during abiotic stress has been documented, including the nucleus, mitochondria, chloroplasts, plasma membrane, and cell wall. ROS may act as signaling molecules in organelles and compartments, based on the distribution of scavenger proteins throughout the cell (Bae et al., 2003; Qiu et al., 2004; Kotchoni et al., 2006; Giacomelli et al., 2006; Komatsu et al., 2009; Nanjo et al., 2010).

#### **13.4.4 OSMOPROTECTANT REGULATORS**

Osmolyte molecules like sugar, mannitol, or amino acids (proline) and their N-methyl derivatives can be controlled by osmoprotectant regulator proteins (betaines). The solutes raise the cell's osmotic pressure, preventing further water loss and preserving turgor (McNeil et al., 1999). Plant responses to abiotic stress have indicated that the expression of genes for sugar syntheses and sugar transporters is frequently up-regulated during abiotic stress, and that buildup of proline and glycine-betaine helps plants overcome the stressed condition, improving tolerance capacity. Commonly osmoprotectants were found majorly in the cytoplasm and all organelles except vacuoles (Maruyama et al., 2004; Ashraf et al., 2006; Aghaei et al., 2009). Soybeans under osmotic stress and stress affecting the ER have been found to have the osmoprotectant biosynthetic protein 1-pyrroline-5-carboxylate synthase, as well as other enzymes including sucrose synthase and sugar transporters. Under stress, osmoprotectants, and water channels are typically responsible for maintaining cell water content.

#### **13.4.5 ION TRANSPORTER PROTEINS**

The changes in the passage of  $H^+$ ,  $K^+$ ,  $Cl^-$ , and  $Ca^{2+}$  ions via the plasma membrane impact cytosolic pH and transmembrane electrical potential. Accumulation of these ions in plant tissues might cause stress.  $Na^+$  and  $Cl^-$  ions are usually retained at low concentrations in plant cells, whereas nutritionally essential components like  $K^+$  are stored at high quantities (Munns & Tester, 2008; Sun et al., 2009). Ion balance is maintained by the plasma membrane and vacuoles, which are two important subcellular components of cells. Regulator proteins, tonoplast  $Na^+/H^+$  antiporters, and plasma membrane  $Na^+/H^+$  antiporters all play a role in adsorbing  $Na^+$  in vacuoles or extruding it to the external environment (Xiong & Zhu, 2002; Waditee et al., 2006). Under drought stress, Proton pumps coupled with the cell membrane regulate the outflow of  $K^+$  and other anions from guard cells that facilitates stomatal closure.  $Ca^{2+}$  ions are involved in maintaining the outflow of anions by regulating the activity of plasma membrane  $H^+$ -ATPase (Kinoshita et al., 1995). Plasma membrane proton pumps are essential membrane proteins that help the cell maintain its ion balance during osmotic stress. Because intracellular ion homeostasis is critical for the functioning of many cellular enzymes, effective ion efflux control is essential during stressful conditions (Hossain et al., 2012).



### **13.4.6 WATER CHANNELS**

Because water is involved in so many cells signaling, biological pathways, and changes in the cell's water content have a negative impact on the cell's operations. Aquaporins (AQP) (plasma membrane and vacuoles) are water channel proteins that allow water and small neutral solutes to pass across the cell membrane (Maurel & Chrispeels, 2001; Vera-Estrella et al., 2004). Plasma membrane-intrinsic AQP and tonoplast-intrinsic AQP are the two types of AQP found in plant cells based on their location and sequence homology. Tonoplast has a high-water permeability; the vacuolar space can act as a buffer against osmotic variations. When a cell is exposed to an excessive amount of water, such as flooding or submersion, the cytoplasmic pH drops, causing protonation of the conserved aquaporin residue (Fischer & Kaldenhoff, 2008). The principal regulators of water balance in plant cells are water channel proteins in the tonoplast and plasma membrane, and water-related stress is the most prevalent and significant environmental factor influencing plants. If a plant cell is exposed to water-related stress, these proteins regulate its water content to ensure that proteins and enzymes in organelles and other cellular compartments continue to function properly.

### **13.5 FUTURE PERSPECTIVE**

In agriculture, abiotic stress influences the potential yield decrease in the crop plants which encourages the study related to how plants respond towards the abiotic stress. The influence of numerous abiotic stresses on the plant cell organelle proteome is discussed in this chapter. Future efforts are made to discover and describe such organelle proteins, which might open up a new path for abiotic stress research based on the proteome. Finally, we hope that this chapter has provided fresh perspectives into plant stress responsiveness, which will be useful in the future development of genetically altered stress-tolerant crop plants for human benefit, as well as emphasizing the importance of examining levels of protein abundance within cellular organelles in response to abiotic stress.

### **ACKNOWLEDGMENT**

The author K. Rakkammal thank the RUSA 2.0 scheme in the form of PhD fellowship [Grant No. F. 24-51/2014-U, Policy (TN Multi-Gen), Department

of Education, Government of India]. The authors also thankfully acknowledge DST-FIST (Grant No. SR/FST/LSI-639/2015(C)), UGC-SAP (Grant No. F. 5-1/2018/DRSII (SAP-II)), and DST-PURSE (Grant No. SR/PURSE Phase 2/38(G)) for providing instrumentation facilities.

## KEYWORDS

- **abiotic stress**
- **ascorbate peroxidase**
- **cell organelle**
- **endoplasmic reticulum**
- **laser capture microdissection**
- **proteomics**

## REFERENCES

- Abdalla, K. O., Baker, B., & Rafudeen, M. S., (2010). Proteomic analysis of nuclear proteins during dehydration of the resurrection plant *Xerophyta viscosa*. *Plant Growth Regulation*, 62, 279–292.
- Aghaei, K., Ehsanpour, A. A., Shah, A. H., & Komatsu, S., (2009). Proteome analysis of soybean hypocotyl and root under salt stress. *Amino Acids*, 36, 91–98.
- Agrawal, G. K., Bourguignon, J., Rolland, N., Ephritikhine, G., Ferro, M., Jaquinod, M., Alexiou, K. G., et al., (2011). Plant organelle proteomics: Collaborating for optimal cell function. *Mass Spectrometry Reviews*, 30, 772–853.
- Ahsan, N., Donnart, T., Nouri, M. Z., & Komatsu, S., (2010b). Tissue specific defense and thermo-adaptive mechanisms of soybean seedlings under heat stress revealed by proteomic approach. *Journal of Proteome Research*, 9, 4189–4204.
- Ahsan, N., Nanjo, Y., Sawada, H., Kohno, Y., & Komatsu, S., (2010a). Ozone stress-induced proteomic changes in leaf total soluble and chloroplast proteins of soybean reveal that carbon allocation is involved in adaptation in the early developmental stage. *Proteomics*, 10, 2605–2619.
- Alexandersson, E., Saalbach, G., Larsson, C., & Kjellbom, P., (2004). *Arabidopsis* plasma membrane proteomics identifies components of transport, signal transduction and membrane trafficking. *Plant and Cell Physiology*, 45, 1543–1556.
- Apel, K., & Hirt, H., (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, 55, 373–399.
- Ashraf, M., & Foolad, M. R., (2006). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, 59, 206–216.

- Bae, M. S., Cho, E. J., Choi, E. Y., & Park, O. K., (2003). Analysis of the *Arabidopsis* nuclear proteome and its response to cold stress. *The Plant Journal*, 36, 652–663.
- Blokhina, O., Virolainen, E., & Fagerstedt, K. V., (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany*, 91, 179–194.
- Bohler, S., Sergeant, K., Hoffmann, L., Dizengremel, P., Hausman, J. F., Renaut, J., & Jolivet, Y., (2011). A difference gel electrophoresis study on thylakoids isolated from poplar leaves reveals a negative impact of ozone exposure on membrane proteins. *Journal of Proteome Research*, 10, 3003–3011.
- Cascardo, J. C., Almeida, R. S., Buzeli, R. A., Carolino, S. M., Otoni, W. C., & Fontes, E. P., (2000). The phosphorylation state and expression of soybean BiP isoforms are differentially regulated following abiotic stresses. *Journal of Biological Chemistry*, 275, 14494–14500.
- Chen, X., Wang, Y., Li, J., Jiang, A., Cheng, Y., & Zhang, W., (2009). Mitochondrial proteome during salt stress-induced programmed cell death in rice. *Plant Physiology and Biochemistry*, 47, 407–415.
- Choudhary, M. K., Basu, D., Datta, A., Chakraborty, N., & Chakraborty, S., (2009). Dehydration-responsive nuclear proteome of rice (*Oryza sativa* L.) illustrates protein network, novel regulators of cellular adaptation, and evolutionary perspective. *Molecular & Cellular Proteomics*, 8, 1579–1598.
- Couee, I., Sulmon, C., Gouesbet, G., & El Amrani, A., (2006). Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *Journal of Experimental Botany*, 57, 449–459.
- De Michele, R., McFarlane, H. E., Parsons, H. T., Meents, M. J., Lao, J., González Fernández-Niño, S. M., Petzold, C. J., et al., (2016). Free-flow electrophoresis of plasma membrane vesicles enriched by two-phase partitioning enhances the quality of the proteome from *Arabidopsis* seedlings. *Journal of Proteome Research*, 15, 900–913.
- Fischer, M., & Kaldenhoff, R., (2008). On the pH regulation of plant aquaporins. *J. Biol. Chem.*, 283, 33889–33892.
- Furman, M. H., Loureiro, J., Ploegh, H. L., & Tortorella, D., (2003). Ubiquitinylation of the cytosolic domain of a type I membrane protein is not required to initiate its dislocation from the endoplasmic reticulum. *Journal of Biological Chemistry*, 278, 34804–34811.
- Giacomelli, L., Rudella, A., & Van, W. K. J., (2006). High light response of the thylakoid proteome in *Arabidopsis* wild type and the ascorbate deficient mutant vtc2-2. A comparative proteomics study. *Plant Physiology*, 141, 685–701.
- Goulas, E., Schubert, M., Kieselbach, T., Kleczkowski, L. A., Gardeström, P., Schröder, W., & Hurry, V., (2006). The chloroplast lumen and stromal proteomes of *Arabidopsis thaliana* show differential sensitivity to short- and long-term exposure to low temperature. *The Plant Journal*, 47, 720–734.
- Hieng, B., Ugrinovic, K., Sustar-Vozlic, J., & Kidric, M., (2004). Different classes of proteases are involved in the response to drought of *Phaseolus vulgaris* L. cultivars differing in sensitivity. *Journal of Plant Physiology*, 161, 519–530.
- Hossain, Z., Lopez-Climent, M. F., Arbona, V., Perez-Clemente, R. M., & Gomez-Cadenas, A., (2009). Modulation of the antioxidant system in citrus under waterlogging and subsequent drainage. *Journal of Plant Physiology*, 166, 1391–1404.
- Hossain, Z., Nouri, M. Z., & Komatsu, S., (2012). Plant cell organelle proteomics in response to abiotic stress. *Journal of Proteome Research*, 11, 37–48.
- Hynek, R., Svensson, B., Jensen, O. N., Barkholt, V., & Finnie, C., (2009). The plasma membrane proteome of germinating barley embryos. *Proteomics*, 9, 3787–3794.

- Irsigler, A. S., Costa, M. D., Zhang, P., Reis, P. A., Dewey, R. E., Boston, R. S., & Fontes, E. P., (2007). Expression profiling on soybean leaves reveals integration of ER and osmotic-stress pathways. *BMC Genomics*, 8, 1–15.
- Jacoby, R. P., Millar, A. H., & Taylor, N. L., (2010). Wheat mitochondrial proteomes provide new links between antioxidant defense and plant salinity tolerance. *Journal of Proteome Research*, 9, 6595–6604.
- Kamal, A. H. M., Cho, K., Kim, D. E., Uozumi, N., Chung, K. Y., Lee, S. Y., Choi, J. S., et al., (2012). Changes in physiology and protein abundance in salt-stressed wheat chloroplasts. *Molecular Biology Reports*, 39, 9059–9074.
- Kawamura, Y., & Uemura, M., (2003). Mass spectrometric approach for identifying putative plasma membrane proteins of *Arabidopsis* leaves associated with cold acclimation. *The Plant Journal*, 36, 141–154.
- Kessler, A., & Brand, M. D., (1994). Quantitative determination of the regulation of oxidative phosphorylation by cadmium in potato tuber mitochondria. *European Journal of Biochemistry*, 225, 923–935.
- Kinoshita, T., Nishimura, M., & Shimazaki, K. I., (1995). Cytosolic concentration of  $\text{Ca}^{2+}$  regulates the plasma membrane  $\text{H}^{+}$ -ATPase in guard cells of fava bean. *The Plant Cell*, 7, 1333–1342.
- Komatsu, S., Kobayashi, Y., Nishizawa, K., Nanjo, Y., & Furukawa, K., (2010). Comparative proteomics analysis of differentially expressed proteins in soybean cell wall during flooding stress. *Amino Acids*, 39, 1435–1449.
- Komatsu, S., Wada, T., Abalea, Y., Nouri, M. Z., Nanjo, Y., Nakayama, N., Shimamura, S., Yamamoto, R., Nakamura, T., & Furukawa, K., (2009). Analysis of plasma membrane proteome in soybean and application to flooding stress response. *Journal of Proteome Research*, 8, 4487–4499.
- Komatsu, S., Yamamoto, A., Nakamura, T., Nouri, M. Z., Nanjo, Y., Nishizawa, K., & Furukawa, K., (2011). Comprehensive analysis of mitochondria in roots and hypocotyls of soybean under flooding stress using proteomics and metabolomics techniques. *Journal of Proteome Research*, 10, 3993–4004.
- Kontunen-Soppela, S., Ossipov, V., Ossipov, S., & Oksanen, E., (2007). Shift in birch leaf metabolome and carbon allocation during long-term open-field ozone exposure. *Global Change Biology*, 13, 1053–1067.
- Kosmala, A., Perlikowski, D., Pawłowicz, I., & Rapacz, M., (2012). Changes in the chloroplast proteome following water deficit and subsequent watering in a high- and a low-drought-tolerant genotype of *Festuca arundinacea*. *Journal of Experimental Botany*, 63, 6161–6172.
- Kotchoni, S. O., & Gachomo, E. W., (2006). The reactive oxygen species network pathways: An essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. *J. Biosci.*, 31, 389–404.
- Kruft, V., Eubel, H., Jansch, L., Werhahn, W., & Braun, H. P., (2001). Proteomic approach to identify novel mitochondrial proteins in *Arabidopsis*. *Plant Physiology*, 127, 1694–1710.
- Kubo, T., Fujita, M., Takahashi, H., Nakazono, M., Tsutsumi, N., & Kurata, N., (2013). Transcriptome analysis of developing ovules in rice isolated by laser microdissection. *Plant and Cell Physiology*, 54, 750–765.
- Le Gall, H., Philippe, F., Domon, J. M., Gillet, F., Pelloux, J., & Rayon, C., (2015). Cell wall metabolism in response to abiotic stress. *Plants*, 4, 112–166.

- Le Sage, V., Cinti, A., & Mouland, A. J., (2016). Proximity-dependent biotinylation for identification of interacting proteins. *Current Protocols in Cell Biology*, 73, 17–19.
- Lilley, K. S., & Dupree, P., (2007). Plant organelle proteomics. *Current Opinion in Plant Biology*, 10, 594–599.
- Longuespee, R., Fleron, M., Pottier, C., Quesada-Calvo, F., Meuwis, M. A., Baiwir, D., Smargiasso, N., et al., (2014). Tissue proteomics for the next decade? Towards a molecular dimension in histology. *Omics: A Journal of Integrative Biology*, 18, 539–552.
- Maruyama, K., Sakuma, Y., Kasuga, M., Ito, Y., Seki, M., Goda, H., Shimada, Y., et al., (2004). Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two microarray systems. *The Plant Journal*, 38, 982–993.
- Maurel, C., & Chrispeels, M. J., (2001). Aquaporins. A molecular entry into plant water relations. *Plant Physiol.*, 125, 135–138.
- McNeil, S. D., Nuccio, M. L., & Hanson, A. D., (1999). Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. *Plant Physiology*, 120, 945–949.
- Millar, A. H., Heazlewood, J. L., Kristensen, B. K., Braun, H. P., & Møller, I. M., (2005). The plant mitochondrial proteome. *Trends in Plant Science*, 10, 36–43.
- Mitoma, J. Y., & Ito, A., (1992). The carboxy-terminal 10 amino acid residues of cytochrome b5 are necessary for its targeting to the endoplasmic reticulum. *The EMBO Journal*, 11, 4197–4203.
- Morkunaite-Haimi, S., Vinskiene, J., Staniene, G., & Haimi, P., (2018). Efficient isolation of chloroplasts from *in vitro* shoots of malus and prunus. *Zemdirbyste-Agriculture*, 105, 171–176.
- Munns, R., & Tester, M., (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59, 651–681.
- Nanjo, Y., Skultety, L., Ashraf, Y., & Komatsu, S., (2010). Comparative proteomic analysis of early-stage soybean seedlings responses to flooding by using gel and gel-free techniques. *Journal of Proteome Research*, 9, 3989–4002.
- Nouri, M. Z., & Komatsu, S., (2010). Comparative analysis of soybean plasma membrane proteins under osmotic stress using gel-based and LC-MS/MS based proteomics approaches. *Proteomics*, 10, 1930–1945.
- Pandey, A., Chakraborty, S., Datta, A., & Chakraborty, N., (2008). Proteomics approach to identify dehydration responsive nuclear proteins from chickpea (*Cicer arietinum* L.). *Molecular & Cellular Proteomics*, 7, 88–107.
- Petrovska, B., Jerabkova, H., Chamrad, I., Vrana, J., Lenobel, R., Urinovska, J., Sebela, M., & Dolezel, J., (2014). Proteomic analysis of barley cell nuclei purified by flow sorting. *Cytogenet Genome Res.*, 143(1–3), 78–86.
- Pitkanen, L., Tuomainen, P., & Eskelin, K., (2014). Analysis of plant ribosomes with asymmetric flow field-flow fractionation. *Analytical and Bioanalytical Chemistry*, 406, 1629–1637.
- Printz, B., Morais, R. D., Wienkoop, S., Sergeant, K., Lutts, S., Hausman, J. F., & Renaut, J., (2015). An improved protocol to study the plant cell wall proteome. *Frontiers in Plant Science*, 6, 237.
- Qiu, Q. S., Guo, Y., Quintero, F. J., Pardo, J. M., Schumaker, K. S., & Zhu, J. K., (2004). Regulation of vacuolar  $\text{Na}^+/\text{H}^+$  exchange in *Arabidopsis thaliana* by the salt-overly-sensitive (SOS) pathway. *Journal of Biological Chemistry*, 279, 207–215.

- Rhee, H. W., Zou, P., Udeshi, N. D., Martell, J. D., Mootha, V. K., Carr, S. A., & Ting, A. Y., (2013). Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. *Science*, 339, 1328–1331.
- Salvato, F., Havelund, J. F., Chen, M., Rao, R. S. P., Rogowska-Wrzesinska, A., Jensen, O. N., Gang, D. R., et al., (2014). The potato tuber mitochondrial proteome. *Plant Physiology*, 164, 637–653.
- Schutzendubel, A., & Polle, A., (2002). Plant responses to abiotic stresses: Heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany*, 53, 1351–1365.
- Senkler, J., Senkler, M., Eubel, H., Hildebrandt, T., Lengwenus, C., Schertl, P., Schwarzlander, M., et al., (2017). The mitochondrial complexome of *Arabidopsis thaliana*. *The Plant Journal*, 89, 1079–1092.
- Smiri, M., Chaoui, A., & El Ferjani, E., (2009). Respiratory metabolism in the embryonic axis of germinating pea seed exposed to cadmium. *Journal of Plant Physiology*, 166, 259–269.
- Sommer, T., & Wolf, D. H., (1997). Endoplasmic reticulum degradation: Reverse protein flow of no return. *The FASEB Journal*, 11, 1227–1233.
- Subba, P., Kumar, R., Gayali, S., Shekhar, S., Parveen, S., Pandey, A., Datta, A., et al., (2013). Characterization of the nuclear proteome of a dehydration-sensitive cultivar of chickpea and comparative proteomic analysis with a tolerant cultivar. *Proteomics*, 13, 1973–1992.
- Sun, J., Chen, S. L., Dai, S. X., Wang, R. G., Li, N. Y., Shen, X., Zhou, X. Y., et al., (2009). Ion flux profiles and plant ion homeostasis control under salt stress. *Plant Signaling & Behavior*, 4, 261–264.
- Tamburino, R., Vitale, M., Ruggiero, A., Sassi, M., Sannino, L., Arena, S., Costa, A., et al., (2017). Chloroplast proteome response to drought stress and recovery in tomato (*Solanum lycopersicum* L.). *BMC Plant Biology*, 17, 1–14.
- Tan, Y. F., O'Toole, N., Taylor, N. L., & Millar, A. H., (2010). Divalent metal ions in plant mitochondria and their role in interactions with proteins and oxidative stress-induced damage to respiratory function. *Plant Physiology*, 152, 747–761.
- Tanaka, K., & Chiba, T., (1998). The proteasome: A protein-destroying machine. *Genes to Cells*, 3, 499–510.
- Taylor, N. L., Heazlewood, J. L., Day, D. A., & Millar, A. H., (2005). Differential impact of environmental stresses on the pea mitochondrial proteome. *Molecular & Cellular Proteomics*, 4, 1122–1133.
- Taylor, N. L., Tan, Y. F., Jacoby, R. P., & Millar, A. H., (2009). Abiotic environmental stress-induced changes in the *Arabidopsis thaliana* chloroplast, mitochondria and peroxisome proteomes. *Journal of Proteomics*, 72, 367–378.
- Vera-Estrella, R., Barkla, B. J., Bohnert, H. J., & Pantoja, O., (2004). Novel regulation of aquaporins during osmotic stress. *Plant Physiology*, 135, 2318–2329.
- Voxeur, A., & Hofte, H., (2016). Cell wall integrity signaling in plants: “to grow or not to grow that’s the question”. *Glycobiology*, 26, 950–960.
- Waditee, R., Tanaka, Y., & Takabe, T., (2006). Na<sup>+</sup>/H<sup>+</sup> antiporters in plants and cyanobacteria. In: Rai, A. K., & Takabe, T., (eds.), *Abiotic Stress Tolerance in Plants: Toward the Improvement of Global Environment and Food* (pp. 163–175). Springer: The Netherlands.
- Wang, W., Vinocur, B., Shoseyov, O., & Altman, A., (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in Plant Science*, 9, 244–252.

- Wiedner, S. D., Anderson, L. N., Sadler, N. C., Chrisler, W. B., Kodali, V. K., Smith, R. D., & Wright, A. T., (2014). Organelle-specific activity-based protein profiling in living cells. *Angewandte Chemie.*, 126, 2963–2966.
- Williams, D. B., (2006). Beyond lectins: The calnexin/calreticulin chaperone system of the endoplasmic reticulum. *Journal of Cell Science*, 119, 615–623.
- Xiong, L., & Zhu, J. K., (2002). Molecular and genetic aspects of plant responses to osmotic stress. *Plant, Cell & Environment*, 25, 131–139.
- Yasueda, Y., Tamura, T., Fujisawa, A., Kuwata, K., Tsukiji, S., Kiyonaka, S., & Hamachi, I., (2016). A set of organelle-localizable reactive molecules for mitochondrial chemical proteomics in living cells and brain tissues. *Journal of the American Chemical Society*, 138, 7592–7602.

## CHAPTER 14

---

# Advances in Metabolomics Research in Environmental Stress Response in Plants

ANKUR SINGH and ARYADEEP ROYCHOUDHURY\*

*Post-Graduate Department of Biotechnology, St. Xavier's College  
(Autonomous), 30, Mother Teresa Sarani, Kolkata – 700016,  
West Bengal, India*

*\*Corresponding author. E-mail: aryadeep.rc@gmail.com*

---

### ABSTRACT

Abiotic stress is one of the major limiting factors that significantly hampers the development of plants and also negatively affects the quality and quantity of crop yield. To overcome the negative effects of abiotic stresses, plants reprogram their metabolic pathway which in turn enhances the level of primary and secondary metabolites. Metabolomics is an important technique for deciphering the unknown metabolites or quantification of known metabolites in plants formed on being exposed to such limiting factors. Metabolome refers to the total metabolite constituent of any plant species that can be analyzed to further explore the changes that take place in plants on being exposed to environmental stresses. Metabolomics is a promising tool that helps in the proper identification of metabolic networks linked with abiotic stress that might help in the identification of stress-specific marker in plants. Advanced metabolomics tools such as non-destructive nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GS-MS), liquid chromatography-mass spectrometry (LC-MS) and direct flow injection mass spectrometry have enhanced the rate of metabolome profiling of plants. Presently, integration of metabolomics with other omics



tools such as genomics and transcriptomics have further enabled the dissection of genetic and phenotypic changes occurring in plants on being exposed to unfavorable environmental conditions. In this chapter, we try to decipher the major role played by metabolomics tools for crop improvement. Here, we describe the workflow of plant metabolomics research, focusing on the elucidation of biotic and abiotic stress tolerance mechanisms in plants along with its potential future role in plant sciences.

## **14.1 INTRODUCTION**

Metabolomics is one of the upcoming and most fascinating tools of omics techniques that have extensively contributed towards the development of crops which resulted in higher survival ability of plants on being exposed to unfavorable environmental conditions. Metabolomics helps us to decipher the total metabolite pool of any plant, which is further measured to characterize the genetic changes occurring in plants in response to abiotic or biotic stresses and metabolic assisted breeding. Till date, around 2,00,000 metabolites have been identified in plants and a wide variety of metabolites is still unknown (Razzaq et al., 2019). All the identified metabolites vary in their structure and function and play a critical role in plant biology. Metabolome profile of an organism refers to all the low weight metabolites. According to Deborde et al. (2017), metabolomics study is mostly linked with identifications and quantifications of small molecules and their functions and interactions with other metabolites in an organism. Most of the metabolites identified in plants are associated with their growth and development, crop yield and tolerance capability against stressed environment (Han & Micallef, 2016). Ecological metabolomics is one of the major branches of metabolomics that help us to understand the interconnection between plant biochemical and various spatial and temporal systems. Ecological metabolome study helps to identify any change in the metabolites level or new metabolites formed in plants in response to abiotic/biotic stresses. Additionally, it also explains the biochemical changes that occur in plants in response to biotic stresses such as infection, attack of parasite or disease occurrence. According to Garcia-Cela et al. (2018), study of variations in the concentration of several metabolites can help to decipher the mechanistic network that eventually explains the physiological, biochemical, and phenotypic changes occurring in plants in response to environmental fluctuations.

Environmental stress restricts the growth and development of plants that ultimately affects the quality and quantity of major food crops worldwide. According to Thakur et al. (2010), abiotic factors such as drought, salinity, heavy metal stress and extreme temperature influence the survival rate, biomass, and crop yield of staple food crops by up to 70% which is going to be an important cause of concern for the continuous growing population of the world. Thus, environmental stress can be defined as any change in surrounding factors that negatively affects the growth and development of plants along with alteration in the metabolic profile of plants. Abiotic stresses induce the formation of reactive oxygen and nitrogen species (ROS and RNS) in cells that eventually cause damage to various biomolecules like protein, carbohydrate, lipid, and DNA (Singh et al., 2020). Additionally, excess formation of ROS can also alter the gene expression levels that hamper the normal homeostasis of plants. To scavenge such cytotoxic metabolites, plants produce a large number of protective metabolites. Primary metabolites are involved in the biosynthesis of sugars, amino acids and lipids that ultimately control the glycolysis and tricarboxylic acid cycle in plants which results in the generation of ATP that fulfill the energy requirement of plants, positively regulate the growth and development of plants, and also enhance their survival capability on being exposed to stressed environmental conditions. Additionally, primary metabolites also contribute towards the formation of secondary metabolites such as flavonoids, carotenoids, anthocyanins, atropine, and phytic acids. Secondary metabolites do not play any critical role in the development of plants; however, their role in reducing the negative effects of abiotic stress in plants by scavenging the ROS in plant tissues is widely studied (Dawid & Hille, 2018). According to Razzaq et al. (2019), some specialized secondary metabolites formed in plants are alkaloids (~21,000), phenolics (~10,000) and terpenoids (>25,000) that enhance the tolerance capability of plants against both abiotic and biotic stress.

In the past, various methods and research knowledge have been applied for identification and detection of both primary and secondary metabolites formed in plants; however, massive production, diverse chemical structures and complex nature of metabolites are the major challenges faced by metabolomics tools during identification of whole metabolome profile (Wishart, 2011). According to Piasecka et al. (2019), advanced techniques for deciphering the complete metabolome profile of any plant species is still in the non-trivial phase and a combination of various techniques comprising of metabolite extraction and various tools for analysis of metabolites are most often used. Additionally, integration of metabolomics with other

omics techniques like genomics and genome-wide sequencing can open a new horizon for the development of stress tolerance in plants. Thus, this chapter describes the advantages of metabolomics in enhancing the survival capability of plants on being exposed to stressed environment. Initially, we briefly describe about the advanced tools used in metabolomics research, followed by the application of metabolomics in plant sciences.

## **14.2 TOOLS USED IN METABOLOMICS**

Choice of any metabolomics approach depends on its accuracy, speed, precision, selectivity, and sensitivity of analytical tools. Performing the complete metabolomics study of plants is a challenging task due to the absence of sufficient information about the connection between proteomics and metabolomics of plants. Along with this, lack of standard protocol, technical hurdles and volatile nature of plant metabolites further worsen the analysis (Zivy et al., 2015). Due to such hurdles, no single technique can provide the full metabolomics profile of any plant so that the combination of various techniques likes nuclear magnetic resonance (NMR), capillary electrophoresis mass spectrometry (CE-MS), liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GS-MS), high performance thin layer chromatography (HPTLC), direct infusion mass spectrometry (DIMS) and Fourier transform ion cyclotron resonance mass spectrometry (FI-ICR-MS) have been extensively used for metabolomics analysis. NMR is one of the most common and widely used techniques for studying the metabolome profile of plants due to its non-destructive, selective, quick, and convenient nature. It is also very proficient in mapping the metabolic pathways (Boiteau et al., 2018). Ma et al. (2018) stated that the metabolic change occurring in plants in response to abiotic/biotic stresses can be monitored using NMR techniques. Moreover, NMR can also detect the structural components of any unidentified metabolites. Recent development in NMR techniques has enabled the researchers to detection of specific labels present on the stable isotopes (Deborde et al., 2017). NMR is a rapid, non-invasive, non-biased, and highly quantitative approach for sample preparation without using chromatographic separation techniques. It helps to probe compounds that are associated with insoluble polymers via solid-state high-resolution NMR (Kim & Choi, 2011). However, lower resolution, poor sensitivity and lower dynamic range has limited the use of NMR.

Challenges faced during the analysis of metabolites of plants using NMR techniques can be easily solved by using MS techniques. Mass spectroscopy

allows the examination of samples in their natural state (Kang et al., 2019). Integration of MS along with HPLC and ultra-high performance liquid chromatography (UPLC) provide a wide range for analysis of plant metabolome profile (Theodoridis et al., 2012). Moreover, GC-MS further enhances the importance of mass spectrometry due to its ability to detect volatile and thermally unstable metabolites (Jorge et al., 2016). It can further be utilized for probing primary metabolites such as sugar-phosphates, amino acids, peptides, sugars, alkaloids, organic acids, ketones, and lipids. With recent integration of other highly efficient mass spectroscopy techniques such as matrix-assisted laser desorption ionization (MALDI) and desorption electrospray ionization mass spectrometry (DESI), it is possible to obtain high resolution images that help us to completely understand the distribution of any metabolite in any specific plant tissue or cell (Enomoto et al., 2018). Thus, with all recent advancements, the metabolomics approach has appeared as one of the more versatile strategies for studying the effects of abiotic stress in plants as compared to that of genomics and proteomics approaches.

### **14.3 ELUCIDATION OF DROUGHT STRESS TOLERANCE IN PLANTS**

Water scarcity or drought is an inevitable factor that hampers plant biomass, energy production and grain yield. According to Brodersen et al. (2019); and Abbasi & Abbasi (2010), around 80–95% of fresh biomass of plants is mainly composed of water that plays a pivotal role in various physiological processes of plants such as their metabolism, and growth and development. Due to such wide role of water in plant physiology, some consider drought as one of the main environmental stresses for plants that can eventually hamper the food supply for the continuously growing population of the world in future (Okorie et al., 2019). Passioura & Angus (2010) stated that the appearance of drought stress depends on the uneven and undependable distribution of rainfall, water holding capacity of soil and evapotranspiration rate of that area. Additionally, in some cases, in spite of water availability, plants are unable to uptake that water from the soil, a phenomenon commonly known as pseudo-drought or physiological drought (Salehi-Lisar & Bakhshayeshan-Agdam, 2020). Environmental factors such as drought frequency and intensity, plant growth condition, characteristic of soil and plant species determines the extent of negative effects of drought stress on plants (Zoghi et al., 2019). Symptoms of water scarcity in plants can range from the production of ROS that result in higher membrane lipid peroxidation, reduced photosynthesis, CO<sub>2</sub> uptake, ATP synthesis obstruction and oxidative damages in chloroplast

to even plant death on being exposed to severe water scarcity (Ullah et al., 2017).

Environmental stresses such as drought stress can trigger the accumulation of large array of protective metabolites (Krasensky & Jonak, 2012). According to Patel et al. (2020), the survival ability of plants in the presence of drought stress mostly depends on the appropriate level of primary and secondary metabolites in plants. Analysis of these metabolites can present the key difference between drought tolerant and drought susceptible plant species which can further be utilized for enhancing the tolerance capability of plants (Llanes et al., 2018). Non-targeted or targeted metabolome study can be used to decipher the metabolic changes that occur in plants in response to stressed environment. Non-targeted metabolomics study helps to understand an overall change in the level of metabolites in plants, whereas targeted metabolomics study detects, analyze, and estimate the level of any known metabolites in plants (Roberts et al., 2012; Van Meulebroek et al., 2016).

Drought stress directly hampers the metabolism of plants, thus forcing the plants to reprogram the biosynthesis and transport of primary and secondary metabolites (Ma et al., 2020). Regulation of metabolism is the key step for implicating safeguarding of cellular osmotic potential during drought stress. According to Liu et al. (2014), alteration in molecular pathways, activation of signaling network, transcription, metabolism, and regulatory response improve the resistance of the plants against drought stress. Kim et al. (2010); and Joshi et al. (2016) reported that transcription factors such as NAC, MYB, AP2/ERF, AREB/ABF and bZIP regulate the stomatal movement along with regulating the expression of drought-responsive genes which eventually regulate the metabolic pathways in plants. Thus, it can be mentioned that drought tolerance in plants is a multi-genic trait. Various metabolomics studies have shown the involvement of several metabolites in inducing drought stress tolerance in several plants. On analyzing the leaves of drought stressed *Avena sativa* via GC, Sanchez-Martin et al. (2018) reported that the level of mono-, di-, and triacylglycerol, free fatty acid, linoleic acid, and palmitic acid was induced in drought tolerant cultivar (Patones) whereas no significant change was noted in drought susceptible cultivar, i.e., Flega. They further showed that upon targeted measurements, the level of jasmonic and isoleucine-jasmonic acid was initially enhanced in the tolerant variety as compared to that of the susceptible variety. Similar study was also performed by Wenzel et al. (2015); Lanzinger et al. (2015); and Hein et al. (2016) in *Hordeum vulgare* where they showed that the level of metabolites like mannitol, galactinol, raffinose, myoinositol, putrescine, proline, isocitric

acid,  $\alpha$ -ketoglutaric acid, glycine, aspartate, serine, and other aromatic acids was enhanced in kernels, grains, and fifth leaf of drought-stressed plants. In another study conducted by Do et al. (2013), the amino acids (arginine, proline, and glutamate), polyamines (spermidine, spermine, and putrescine) and  $\gamma$ -aminobutyric acid were involved in the regulation of drought stress tolerance in the leaves of 21 rice cultivars. On analyzing the leaves and roots of *Triticum aestivum* via GC-MS, the levels of amino acids such as valine and tryptophan and other metabolites, i.e., citrate, malate, and fumarate were enhanced and reduced, respectively in the leaf and root of drought tolerant plant (LA754). In contrast, the level of glyceric acid was enhanced in root and reduced in leaf. The results suggested that the metabolic composition of root and shoot respond variably on being exposed to harsh conditions and more variation could be noted in the shoot metabolome as compared to that of root (Kang et al., 2019). Along with this, various metabolome studies have been conducted in plant species such as *Vitis vinifera*, *Glycine max*, *Cicer arietinum*, lentils, *Nicotiana tabacum*, etc., which showed that drought stress significantly alters the metabolome profile of plants (Griesser et al., 2015; Silvente et al., 2012; Khan et al., 2019; Muscolo et al., 2015).

#### **14.4 CONTRIBUTION OF METABOLOME STUDY IN AMELIORATING THE EFFECTS OF SALT STRESS**

Salt can easily mix with both surface and groundwater and thus presence of excess salt in the environment is highly toxic for the growth and development of plants. According to Velmurugan et al. (2020), around 20% (~900 million ha) of land which is estimated to be half of the total cultivable land is contaminated with excess salt concentration. Therefore, salt toxicity is one the major causes of concern for the total food supply of the world. Excess salt stress negatively hampers the morphology and biochemical function of plants. Salt stress also inhibit seed germination, photosynthetic machineries, gaseous exchanges, and transpiration rate, as well as lower the content of carotenoids and chlorophyll, stomatal conductance and disintegrates the structure of photosystem (PS) II and chloroplast (Pan et al., 2020; Zhang & Dai, 2019). Plants uptake excess salt from soil via transporters that disturbs absorption and homeostasis of other necessary ions such as  $K^+$  and  $Ca^{2+}$  along with higher deposition of  $Na^+$  and  $Cl^-$  ions which leads to ionic toxicity in plant tissues (Isayenkov & Maathuis, 2019). El-Ghazail (2020) reported that excess accumulation of  $Na^+$  and  $Cl^-$  ions also enhance the formation of ROS,

which in turn damages the lipid membrane and also causes DNA and protein disintegration.

The plant enhances their survival ability against salt stress through various strategies and approaches such as ion homeostasis and compartmentalization, osmotic adaptation, transport of ions, upregulating the formation of polyamines and antioxidants (de Freitas et al., 2019). Plants adapt themselves by enhancing the formation of protective metabolites which eventually detoxify the cytotoxic metabolites. The study of the metabolome profile of plants exposed to salt stress gives a comprehensive knowledge about the change in the level of these metabolites contributing towards higher tolerance level of plants. Plants respond to salinity stress by enhancing the level of certain amino acid such as phenylalanine, methionine, ornithine, glutamate, dihydroxy isoleucine, L-methionine, L-homomethionine, 3-methoxytyramine, tetra-homomethionine, 1-(3-aminopropyl)-4-aminobutanal, feruloylagmatine, and methionine derivatives, whereas the level of other amino acids like glutamine, cysteine, tyrosine, D-alanyl-D-serine, D-alanyl-D-alanine, L-alanyl-L-glutamate were reduced (Benjamin et al., 2019; Seo et al., 2018; D'Amelia et al., 2018). According to Hildebrandt et al. (2015), the higher level of glycine betaine, proline, and ethanolamine maintains the enzymatic activity and protein structure and also acts as an osmoregulator in plants subjected to severe salt toxicity. The other group of secondary metabolites like flavonoids and phenolic compounds also take part in the amelioration of negative effects of salt stress in plants. Benjamin et al. (2019) reported that the level of flavonoids (dihydroquercetin, 4'-methoxyisoflavone, flavonol 3-O-galactoside, epicatechin-3-O-gallate, and sakuranin) was enhanced in the roots of halophytes on being exposed to salt stress. Additionally, GC-MS based metabolome profiling of salt-stressed rice seedlings showed hyperaccumulation of some major amino acids like valine, leucine, isoleucine, and proline (Gayen et al., 2019). In another study, Gupta & De (2017) demonstrated that the level of two signaling molecules, i.e., serotonin and gentisic acid was enhanced in the seedlings of salt stressed-tolerant rice cultivars (Nonabokra and Bhutnath) as compared to that of the sensitive varieties (MTU, 7029 and Sujala). Thus, these two molecules are important biomarker compounds in the tolerant varieties. Similarly, Chang et al. (2019) reported that the level of mannitol and sucrose was enhanced, whereas the level of shikimate and quinate was lowered in the salt-tolerant rice variety. GC-MS-based approach was followed by Sheldon et al. (2016) to demonstrate the changes in the root metabolome profile of the salt-stressed tolerant barley cultivar (Clipper) and susceptible variety (Sahara). They showed that the root

growth was maintained in the tolerant variety due to higher accumulation of sugars (sucrose, maltose, and xylose), amino acid (proline) and organic acids (shikimate and gluconate), thus indicating the potential role of these metabolites in abrogation of salt toxicity. Additionally, similar reports have also been reported in other plant species such as maize, tomato, wheat, and barley (Zörb et al., 2013; Rouphael et al., 2018; Borrelli et al., 2018).

#### **14.5 METABOLOMICS IN ATTENUATING THE NEGATIVE IMPACT OF EXTREME TEMPERATURE**

Temperature is one the major factors that regulates the development of the plants. According to Pyl et al. (2012), plants are continuously exposed to changes in diurnal or seasonal temperature and thus they must continuously regulate their metabolism and physiology to ameliorate the negative effects of extreme (higher or lower) temperature. On being exposed to either heat or cold stress, plants can experience a severe degree of cellular, physiological, molecular, and metabolic dysfunction (Guy et al., 2008). Plants on being exposed to short-term cold stress become tolerant to freezing temperature via cold acclimation. Similarly, earlier exposure to short-term heat stress also induces the tolerance capability of plants to higher temperature known as acquired tolerance which requires extensive metabolic reprogramming (Espinoza et al., 2010; Caldana et al., 2011).

Various metabolome studies have shown that plants adapt themselves according to the changes occurring in the surrounding environment by altering their metabolite profile. Dhatt et al. (2019) showed that heat stress altered the expression of 19 metabolites out of 57 metabolites as detected by GC-MS in rice seedlings. They further reported that the level of sugar molecules (glucose, fructose, and sucrose), starch biosynthesis and tricarboxylic acid cycle (TCA) were all enhanced in heat-stressed rice plants during the early phase of grain filling. Recently, Wang et al. (2020) using GC-MS technique, showed that the level of soluble sugar was enhanced in heat-shocked *Arabidopsis thaliana*, whereas the plants which were exposed to prolonged warming showed higher content of sorbitol and reduced level of fumaric acid, citric acid, and L-malic acid. Thus, they inferred that plants respond to heat by enhancing transpiration, photosynthesis, and respiratory electron transfer, whereas on being exposed to prolonged heat, plants generally lower stomatal conduction and TCA cycle and increase photosynthetic electron transfer rate. Similarly, Ren et al. (2019) analyzed the leaves of *Populus tomentosa* using GC-MS and reported that the level of proline,



melibiose, and raffinose was induced during heat stress which eventually enhanced glycolytic pathways along with significant increase in the level of amino acids (valine, leucine, and arginine).

In addition to high temperature, temperature, lower than the optimum level can also cause significant damage to the plants. Using HPLC and NMR, Chen et al. (2020) reported that cold stress significantly altered the level of metabolites in banana. They demonstrated that cold stress enhances the level of proline, leucine, isoleucine, acetic acid, malic acid, linoleic acid, and sucrose that contributed toward higher stress tolerance in plants. Similarly, Jian et al. (2020) reported that 47 and 41 metabolites were significantly regulated in the leaves of cold stressed winter and spring oilseed, respectively. Of all the metabolites identified, 83 metabolites were directed toward primary metabolism. Secondary metabolic pathways like flavonoid biosynthesis, phenylpropanoid biosynthesis, flavone and flavonol biosynthesis, and carotenoid biosynthesis also contributed toward stress tolerance in plants. Seeds of the cold stressed rice plants on being analyzed via HPLC and LC-MS/MS showed that the level of 35 metabolites out of 730 metabolites were significantly altered that mostly participated in phenylpropanoid and amino acid metabolism pathways (Yang et al., 2019). Additionally, the effects of cold stress in the metabolome of other plant species such as *Nicotiana tabacum*, *Vitis amurensis*, *Vitis vinifera*, *Hordeum distichon* and *Triticum aestivum* have been reported which showed that plants significantly change their metabolic pathway on being exposed to lower temperature (Xu et al., 2020; Chai et al., 2019; Yang et al., 2020; Cheong et al., 2019).

## 14.6 HEAVY METAL INDUCED ALTERATION IN METABOLOME OF PLANTS

During the past few decades, increased anthropogenic activities, modern agricultural norms and rapid industrialization have drastically increased the level of heavy metals in soil that causes toxicity to the plants (Miransari, 2011; Kavamura & Exposito, 2010). According to Jarup (2003), 53 elements belonging to the d-block of the periodic table has been recognized as heavy metals due to their density ( $> 5 \text{ g cm}^{-3}$ ). Of all the elements known, carbon (C), oxygen (O), hydrogen (H), magnesium (Mg), sulfur (S), nitrogen (N), calcium (Ca), phosphorus (P), and potassium (K) are regarded as macronutrients. Copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), molybdenum (Mo), boron (B), nickel (Ni), cobalt (Co), and chlorine (Cl) are considered

as micronutrients that are required for survival of plants. Apart from these elements, heavy metals such as cadmium (Cd), chromium (Cr), aluminum (Al), lead (Pb) and mercury (Hg) are also present in soil and are taken up by the plants. Excess accumulation of these heavy metals in plant tissues can lead to severe toxicity and thus their translocation from soil is tightly regulated by the plants (Farias et al., 2013; Fidalgo et al., 2013). Higher deposition of these toxic elements in cells might lead to reduction in biomass and chlorophyll degradation, inhibition in the uptake of water and nutrients, hampering growth and photosynthesis, and enhancing senescence which eventually leads to plant death. Along with hampering the growth and development of plants, heavy metal also negatively affects the health of human and animals. Plants growing in the contaminated metal sites uptake higher amount of toxic metals in their tissues that ultimately enters the food chain and eventually make humans prone to various diseases that includes various types of cancer and dermatitis (McLaughlin et al., 1999).

To combat heavy metal stress, plants need to stabilize their protein structure, synchronize multi-dimensional physiological and biochemical processes, and also alter their metabolome accordingly for developing metal stress tolerance. Jahangir et al. (2008) showed that on being exposed to heavy metals, the level of glucosinolates and hydroxycinnamic acid was induced in the leaves and roots of canola plants as revealed through NMR. Similar studies were also performed in leaves and roots of Cr and Mo stressed sunflower and soybean plants, respectively (Ibarra et al., 2019; Xu et al., 2018). The level of fatty acid was found to be enhanced in the tissues of Cr-stressed sunflower plants, whereas the level of other metabolites, i.e., citric acid, gluconic acid, L-nicotine, and flavonoids/isoflavone was found to be enhanced in Mo stressed soybean plants. In another study, Manivasagaperumal et al. (2011) showed that the level of carbohydrate was significantly induced in the beans treated with higher concentration of Cu and Zn. Along with above-mentioned metabolites, the level of other metabolites such as  $\alpha$ -tocopherol, glutathione, proline, histidine, and phenolics was also found to be enhanced in plants in response to heavy metal stress (Collin et al., 2008; Singh et al., 2015; Sharma & Dietz, 2006; Kerkeb & Kramer, 2003; Diaz et al., 2001).

## 14.7 CONCLUSION

In this chapter, the metabolomics approach adopted by the research community in ameliorating the negative effects of abiotic stress (drought,

salinity, heat, cold, and heavy metal stress) has been briefly discussed. Before the discovery of various metabolomics techniques, identification of specialized molecules formed in plants in response to abiotic stress was mostly unknown. Metabolomics plays a prominent role in plant biology. Its diverse role that ranges from quantification of the level of known metabolites in plants to identification of unknown smaller weight metabolites formed in plants in response to abiotic stress has become a handy tool for both researchers and plant breeders. However, it is still an early branch of science and various aspects such as data mining and annotation, assessment, processing, and evaluation needs proper attention. Integration of metabolomics data with other omics techniques will proficiently dissect novel metabolic network for enhancing the stress tolerance capability of plants.

In the future, by using metabolomics approach, specific markers can be identified that can eventually be linked with the nature and scale of effects caused by abiotic stress in plants. Approaches like metabolome assisted plant breeding might be very useful for the development of climate resilient plants along with higher yield. Another important topic that needs to be addressed in coming time is higher subcellular compartmentalization. Biosynthesis of metabolites takes place in various organelles and thus more sophisticated tools in sampling highly spatially and temporally resolved tissues will be necessary to unravel the complexity of interacting metabolic pathways. Combining the metabolomics with specific isotope labeling of metabolites can lead to the detection of metabolites in cell organelles along with their metabolic pathway. Taken together, our understanding of metabolite adaptation to abiotic stress in plants is still quite obscure. It is of utmost importance to further enhance our knowledge of metabolite adaptation to abiotic stress with targeted comprehensive metabolomics studies emphasizing primary and secondary metabolic pathways.

## **ACKNOWLEDGMENTS**

Dr. Aryadeep Roychoudhury is highly thankful for the financial assistance received from Department of Higher Education, Science and Technology and Biotechnology, Government of West Bengal and Science and Engineering Research Board (SERB), Government of India for the grant [264(Sanc.)/ST/P/S&T/1G-80/2017] and [EMR/2016/004799], respectively.

## KEYWORDS

- **abiotic stress**
- **compatible solutes**
- **mass spectrometry**
- **metabolomics**
- **primary metabolites**
- **secondary metabolites**

## REFERENCES

- Abbasi, T., & Abbasi, S. A., (2010). Biomass energy and the environmental impacts associated with its production and utilization. *Renew. Sustain. Energy Rev.*, 14, 919–937.
- Benjamin, J. J., Lucini, L., Jothiramshekar, S., & Parida, A., (2019). Metabolomic insights into the mechanisms underlying tolerance to salinity in different halophytes. *Plant Physiol. Biochem. (Paris)*, 135, 528–545.
- Boiteau, R., Hoyt, D., Nicora, C., Kinmonth-Schultz, H., Ward, J., & Bingol, K., (2018). Structure elucidation of unknown metabolites in metabolomics by combined NMR and MS/MS prediction. *Metabolites*, 8, 8.
- Borrelli, G. M., Fragasso, M., Nigro, F., Platani, C., Papa, R., Beleggia, R., & Trono, D., (2018). Analysis of metabolic and mineral changes in response to salt stress in durum wheat (*Triticum turgidum* ssp. durum) genotypes, which differ in salinity tolerance. *Plant Physiol. Biochem.*, 133, 57–70.
- Brodersen, C. R., Roddy, A. B., Wason, J. W., & McElrone, A. J., (2019). Functional status of xylem through time. *Annu. Rev. Plant Biol.*, 70, 407–433.
- Caldana, C., Degenkolbe, T., Cuadros-Inostroza, A., Klie, S., Sulpice, R., Leisse, A., Steinhauser, D., et al., (2011). High-density kinetic analysis of the metabolomic and transcriptomic response of *Arabidopsis* to eight environmental conditions. *Plant J.*, 67, 869–884.
- Chai, F., Liu, W., Xiang, Y., Meng, X., Sun, X., Cheng, C., Liu, G., et al., (2019). Comparative metabolic profiling of *Vitis amurensis* and *Vitis vinifera* during cold acclimation. *Hortic. Res.*, 6, 8.
- Chang, J., Cheong, B. E., Natera, S., & Roessner, U., (2019). Morphological and metabolic responses to salt stress of rice (*Oryza sativa* L.) cultivars which differ in salinity tolerance. *Plant Physiol. Biochem.*, 144, 427–435.
- Chen, L., Zhao, X., Wu, J., He, Y., & Yang, H., (2020). Metabolic analysis of salicylic acid-induced chilling tolerance of banana using NMR. *Food Res. Int.*, 128, 108796.
- Cheong, B. E., Ho, W. W. H., Biddulph, B., Wallace, X., Rathjen, T., Rupasinghe, T. W. T., Roessner, U., & Dolferus, R., (2019). Phenotyping reproductive stage chilling and frost tolerance in wheat using targeted metabolome and lipidome profiling. *Metabolomics*, 15, 144.

- Collin, V. C., Eymery, F., Genty, B., Rey, P., & Havaux, M., (2008). Vitamin E is essential for the tolerance of *Arabidopsis thaliana* to metal-induced oxidative stress. *Plant Cell Environ.*, 31, 244–257.
- D’Amelia, L., Dell’Aversana, E., Woodrow, P., Ciarmiello, L. F., & Carillo, P., (2018). Metabolomics for crop improvement against salinity stress. In: Kumar, V., Wani, S. H., Suprasanna, P., & Tran, L-S. P., (eds.), *Salinity Responses and Tolerance in Plants: Exploring RNAi, Genome Editing and Systems Biology* (Vol. 2, pp. 267–287). Springer International Publishing, Cham.
- Dawid, C., & Hille, K., (2018). Functional metabolomics—A useful tool to characterize stress-induced metabolome alterations opening new avenues towards tailoring food crop quality. *Agronomy*, 8, 138.
- De Freitas, P. A. F., De Carvalho, H. H., Costa, J. H., Miranda, R. S., Saraiva, K., De Oliveira, F. D. B., Coelho, D. G., et al., (2019). Salt acclimation in sorghum plants by exogenous proline: Physiological and biochemical changes and regulation of proline metabolism. *Plant Cell Rep.*, 38, 403–416.
- Deborde, C., Moing, A., Roch, L., Jacob, D., Rolin, D., & Giraudeau, P., (2017). Plant metabolism as studied by NMR spectroscopy. *Prog. Nucl. Magn. Reson. Spectrosc.*, 102, 61–97.
- Dhatt, B. K., Abshire, N., Paul, P., Hasanthika, K., Sandhu, J., Zhang, Q., Obata, T., & Walia, H., (2019). Metabolic dynamics of developing rice seeds under high night-time temperature stress. *Front Plant Sci.*, 10, 1443.
- Diaz, J., Bernal, A., Po Mar, F., & Merino, F., (2001). Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annuum* L.) seedlings in response to copper stress and its relation to lignification. *Plant Sci.*, 161, 179.
- Do, P. T., Degenkolbe, T., Erban, A., Heyer, A. G., Kopka, J., Köhl, K. I., Hinch, D. K., & Zuther, E., (2013). Dissecting rice polyamine metabolism under controlled long-term drought stress. *PLoS One*, 8, e60325.
- El Ghazali, G. E. B., (2020). *Suaeda vermiculata* Forssk. ex J.F. Gmel.: Structural characteristics and adaptations to salinity and drought: A review. *Intermt. J. Sci.*, 9, 28–33.
- Enomoto, H., Sensu, T., Yumoto, E., Yokota, T., & Yamane, H., (2018). Derivatization for detection of abscisic acid and 12-oxo-phytodienoic acid using matrix-assisted laser desorption/ionization imaging mass spectrometry. *Rapid Commun. Mass Spectrom.*, 32, 1565–1572.
- Espinoza, C., Degenkolbe, T., Caldana, C., Zuther, E., Leisse, A., Willmitzer, L., Hinch, D. K., & Hannah, M. A., (2010). Interaction with diurnal and circadian regulation results in dynamic metabolic and transcriptional changes during cold acclimation in *Arabidopsis*. *PLoS One*, 5, e14101.
- Farias, J. G., Antes, F. L. G., Nunes, P. A. A., Nunes, S. T., Schaich, G., Rossato, L. V., Miotto, A., et al., (2013). Effects of excess copper in vineyard soils on the mineral nutrition of potato genotypes. *Food Energy Security*, 2, 49–69.
- Fidalgo, F., Azenha, M., Silva, A. F., De Sousa, A., Santiago, A., Ferraz, P., & Teixeira, J., (2013). Copper-induced stress in *Solanum nigrum* L. and antioxidant defense system responses. *Food Energy Security*, 2, 70–80.
- Garcia-Cela, E., Kiaitsi, E., Medina, A., Sulyok, M., Krska, R., & Magan, N., (2018). Interacting environmental stress factors affect targeted metabolomic profiles in stored natural wheat and that inoculated with *F. graminearum*. *Toxins*, 10, 56.

- Gayen, D., Barua, P., Lande, N. V., Varshney, S., Sengupta, S., Chakraborty, S., & Chakraborty, N., (2019). Dehydration-responsive alterations in the chloroplast proteome and cell metabolomic profile of rice reveals key stress adaptation responses. *Environ. Exper. Bot.*, 160, 12–24.
- Griesser, M., Weingart, G., Schoedl-Hummel, K., Neumann, N., Becker, M., Varmuza, K., Liebner, F., et al., (2015). Severe drought stress is affecting selected primary metabolites, polyphenols, and volatile metabolites in grapevine leaves (*Vitis vinifera* cv. Pinot noir). *Plant Physiol. Biochem.*, 88, 17–26.
- Gupta, P., & De, B., (2017). Metabolomics analysis of rice responses to salinity stress revealed elevation of serotonin, and gentisic acid levels in leaves of tolerant varieties. *Plant Signal Behav.*, 12, e1335845.
- Guy, C. L., Kaplan, F., Kopka, J., Selbig, J., & Hinch, D. K., (2008). Metabolomics of temperature stress. *Physiol. Plantarum*, 132, 220–235.
- Han, S., & Micallef, S. A., (2016). Environmental metabolomics of the tomato plant surface provides insights on *Salmonella enterica* colonization. *Appl. Environ. Microbiol.*, 82, 3131–3142.
- Hein, J. A., Sherrard, M. E., Manfredi, K. P., & Abebe, T., (2016). The fifth leaf and spike organs of barley (*Hordeum vulgare* L.) display different physiological and metabolic responses to drought stress. *BMC Plant Biol.*, 16, 248.
- Hildebrandt, T. M., Nesi, N. A., Araújo, W. L., & Braun, H. P., (2015). Amino acid catabolism in plants. *Mol. Plant.*, 8, 1563–1579.
- Ibarra, A. A. G., Wrobel, K., Barrientos, E. Y., Escobosa, A. R. C., Corona, J. F. G., Donis, I. E., & Wrobel, K., (2019). Impact of Cr (VI) on the oxidation of polyunsaturated fatty acids in *Helianthus annuus* roots studied by metabolomics tools. *Chemosphere*, 220, 442–451.
- Isayenkov, S. V., & Maathuis, F. J. M., (2019). Plant salinity stress: Many unanswered questions remain. *Front Plant Sci.*, 10, 80.
- Jahangir, M., Abdel-Farid, I. B., Choi, Y. H., & Verpoorte, R., (2008). Metal ion-inducing metabolite accumulation in *Brassica rapa*. *J. Plant Physiol.*, 165, 1429–1437.
- Jarup, L., (2003). Hazards of heavy metal contamination. *Br. Med. Bull.*, 68, 167–182.
- Jian, H., Xie, L., Wang, Y., Cao, Y., Wan, M., Lv, D., Li, J., et al., (2020). Characterization of cold stress responses in different rapeseed ecotypes based on metabolomics and transcriptomics analyses. *PeerJ*, 8, e8704.
- Jorge, T. F., Rodrigues, J. A., Caldana, C., Schmidt, R., Van, D. J. T., Thomas-Oates, J., & António, C., (2016). Mass spectrometry-based plant metabolomics: Metabolite responses to abiotic stress. *Mass Spectrom. Rev.*, 35, 620–649.
- Joshi, R., Wani, S. H., Singh, B., Bohra, A., Dar, Z. A., Lone, A. A., Pareek, A., & Singla-Pareek, S. L., (2016). Transcription factors and plants response to drought stress: Current understanding and future directions. *Front Plant Sci.*, 7, 1029.
- Kang, Z., Babar, M. A., Khan, N., Guo, J., Khan, J., Islam, S., Shrestha, S., & Shahi, D., (2019). Comparative metabolomics profiling in the roots and leaves in contrasting genotypes reveals complex mechanisms involved in post-anthesis drought tolerance in wheat. *PLoS One*, 14, e0213502.
- Kavamura, V. N., & Esposito, E., (2010). Biotechnological strategies applied to the decontamination of soils polluted with heavy metal. *Biotechnol. Adv.*, 28, 61–69.
- Kerkeb, L., & Kramer, U., (2003). The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*. *Plant Physiol.*, 131, 716–724.

- Khan, N., Bano, A., Rahman, M. A., & Rathinasabapathi, B., (2019). UPLC-HRMS-based untargeted metabolic profiling reveals changes in chickpea (*Cicer arietinum*) metabolome following long-term drought stress. *Plant Cell Environ.*, 42, 115–132.
- Kim, H. K., Choi, Y. H., & Verpoorte, R., (2011). NMR-based plant metabolomics: Where do we stand, where do we go? *Trends Biotechnol.*, 29, 267–275.
- Kim, T. H., Bohmer, M., Hu, H., Nishimura, N., & Schroeder, J. I., (2010). Guard cell signal transduction network: Advances in understanding abscisic acid, CO<sub>2</sub>, and Ca<sup>2+</sup> signaling. *Annu., Rev. Plant Biol.*, 61, 561–591.
- Krasensky, J., & Jonak, C., (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.*, 63, 1593–1608.
- Lanzinger, A., Frank, T., Reichenberger, G., Herz, M., & Engel, K. H., (2015). Metabolite profiling of barley grain subjected to induced drought stress: Responses of free amino acids in differently adapted cultivars. *J. Agric. Food Chem.*, 63, 4252–4261.
- Liu, J., Chen, N., Chen, F., Cai, B., Dal Santo, S., Tornielli, G. B., Pezzotti, M., & Cheng, Z. M., (2014). Genome-wide analysis and expression profile of the bZIP transcription factor gene family in grapevine (*Vitis vinifera*). *BMC Genom.*, 15, 281.
- Llanes, A., Andrade, A., Alemano, S., & Luna, V., (2018). Metabolomic approach to understand plant adaptations to water and salt stress. In: Ahmad, P., Ahanger, M. A., Singh, V. P., Tripathi, D. K., Alam, P., & Alyemeni, M. N., (eds.), *Plant Metabolites and Regulation Under Environmental Stress* (pp. 133–144). Academic Press: Cambridge, MA, USA.
- Ma, N. L., Lah, W. A. C., Kadir, N. A., Mustaqim, M., Rahmat, Z., Ahmad, A., Lam, S. D., & Ismail, M. R., (2018). Susceptibility and tolerance of rice crop to salt threat: Physiological and metabolic inspections. *PLoS One*, 13, e0192732.
- Ma, X. J., Yu, T. F., Li, X. H., Cao, X. Y., Ma, J., Chen, J., Zhou, Y. B., et al., (2020). Overexpression of *GmNFYA5* confers drought tolerance to transgenic *Arabidopsis* and soybean plants. *BMC Plant Biol.*, 20, 123.
- Manivasagaperumal, R., Balamurugan, S., Thiyagarajan, G., & Sekar, J., (2011). Effect of zinc on germination, seedling growth and biochemical content of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub). *Curr. Bot.*, 2, 11–15.
- McLaughlin, M. J., Parker, D. R., & Clark, J. M., (1999). Metal and micronutrients-food safety issues. *Field Crops Res.*, 60, 143–163.
- Miransari, M., (2011). Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metal. *Biotechnol. Adv.*, 29, 645–653.
- Muscolo, A., Junker, A., Klukas, C., Weigelt-Fischer, K., Riewe, D., & Altmann, T., (2015). Phenotypic and metabolic responses to drought and salinity of four contrasting lentil accessions. *J. Exp. Bot.*, 66, 5467–5480.
- Okorie, V. O., Mphambukeli, T. N., & Amusan, S. O., (2019). Exploring the political economy of water and food security nexus in BRICS. *Afr. Insight*, 48, 21–38.
- Pan, T., Liu, M., Kreslavski, V. D., Zharmukhamedov, S. K., Nie, C., Yu, M., Kuznetsov, V. V., et al., (2020). Non-stomatal limitation of photosynthesis by soil salinity. *Crit. Rev. Environ. Sci. Technol.*, 1–35.
- Passioura, J. B., & Angus, J. F., (2010). Improving productivity of crops in water-limited environments. In: *Advances in Agronomy* (Vol. 106, pp. 37–75). Academic Press: Cambridge, MA, USA.
- Patel, M. K., Kumar, M., Li, W., Luo, Y., Burritt, D. J., Alkan, N., & Tran, L. S. P., (2020). Enhancing salt tolerance of plants: From metabolic reprogramming to exogenous chemical treatments and molecular approaches. *Cells*, 9, 2492.

- Piasecka, A., Kachlicki, P., & Stobiecki, M., (2019). Analytical methods for detection of plant metabolomes changes in response to biotic and abiotic stresses. *Int. J. Mol. Sci.*, 20, 379.
- Pyl, E. T., Piques, M., Ivakov, A., Schulze, W., Ishihara, H., Stitt, M., & Sulpice, R., (2012). Metabolism and growth in *Arabidopsis* depend on the daytime temperature but are temperature-compensated against cool nights. *Plant Cell*, 24, 2443–2469.
- Razzaq, A., Sadia, B., Raza, A., Khalid, H. M., & Saleem, F., (2019). Metabolomics: A way forward for crop improvement. *Metabolites*, 9, 303.
- Ren, S., Ma, K., Lu, Z., Chen, G., Cui, J., Tong, P., Wang, L., et al., (2019). Transcriptomic and metabolomics analysis of the heat-stress response of *Populus tomentosa* carr. *Forests*, 10, 383.
- Roberts, L. D., Souza, A. L., Gerszten, R. E., & Clish, C. B., (2012). Targeted metabolomics. *Curr. Protoc. Mol. Biol.*, 98, 30.
- Rouphael, Y., Raimondi, G., Lucini, L., Carillo, P., Kyriacou, M. C., Colla, G., Cirillo, V., et al., (2018). Physiological and metabolic responses triggered by omeprazole improve tomato plant tolerance to NaCl stress. *Front Plant Sci.*, 9, 249.
- Salehi-Lisar, S. Y., & Bakhshayeshan-Agdam, H., (2020). Agronomic crop responses and tolerance to drought stress. In: *Agronomic Crops* (pp. 63–91). Springer: Berlin/Heidelberg, Germany.
- Sanchez-Martin, J., Canales, F. J., Tweed, J. K. S., Lee, M. R. F., Rubiales, D., Gómez-Cadenas, A., Arbona, V., et al., (2018). Fatty acid profile changes during gradual soil water depletion in oats suggests a role for jasmonates in coping with drought. *Front Plant Sci.*, 9, 1077.
- Seo, S. H., Park, S. E., Kim, E. J., Lee, K. I., Na, C. S., & Son, H. S., (2018). A GC-MS based metabolomics approach to determine the effect of salinity on kimchi. *Food Res. Int.*, 105, 492–498.
- Sharma, S. S., & Dietz, K. J., (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot.*, 57, 711–726.
- Shelden, M. C., Dias, D. A., Jayasinghe, N. S., Bacic, A., & Roessner, U., (2016). Root spatial metabolite profiling of two genotypes of barley (*Hordeum vulgare* L.) reveals differences in response to short-term salt stress. *J. Exp. Bot.*, 67, 3731–3745.
- Silvente, S., Sobolev, A. P., & Lara, M., (2012). Metabolite adjustments in drought tolerant and sensitive soybean genotypes in response to water stress. *PLoS One*, 7, e38554.
- Singh, A., Banerjee, A., & Roychoudhury, A., (2020). Seed priming with calcium compounds abrogate fluoride-induced oxidative stress by upregulating defense pathways in an indica rice variety. *Protoplasma*, 257, 767–782.
- Singh, V. P., Singh, S., Kumar, J., & Prasad, S. M., (2015). Investigating the roles of ascorbate-glutathione cycle and thiol metabolism in arsenate tolerance in ridged luffa seedlings. *Protoplasma*, 252, 1217–1229.
- Thakur, P., Kumar, S., Malik, J. A., Berger, J. D., & Nayyar, H., (2010). Cold stress effects on reproductive development in grain crops: An overview. *Environ. Exp. Bot.*, 67, 429–443.
- Theodoridis, G., Gika, H., Franceschi, P., Caputi, L., Arapitsas, P., Scholz, M., Masuero, D., et al., (2012). LC-MS based global metabolite profiling of grapes: Solvent extraction protocol optimization. *Metabolomics*, 8, 175–185.
- Ullah, A., Sun, H., Yang, X., & Zhang, X., (2017). Drought coping strategies in cotton: Increased crop per drop. *Plant Biotechnol. J.*, 15, 271–284.



- Van, M. L., Hanssens, J., Steppe, K., & Vanhaecke, L., (2016). Metabolic fingerprinting to assess the impact of salinity on carotenoid content in developing tomato fruits. *Int. J. Mol. Sci.*, 17, 821.
- Velmurugan, A., Swarnam, P., Subramani, T., Meena, B., & Kaledhonkar, M. J., (2020). Water demand and salinity. *Desalination – Challenges and Opportunities*. IntechOpen.
- Wang, L., Ma, K. B., Lu, Z. G., Ren, S. X., Jiang, H. R., Cui, J. W., Chen, G., et al., (2020). Differential physiological, transcriptomic and metabolomic responses of *Arabidopsis* leaves under prolonged warming and heat shock. *BMC Plant Biol.*, 20, 86.
- Wenzel, A., Frank, T., Reichenberger, G., Herz, M., & Engel, K. H., (2015). Impact of induced drought stress on the metabolite profiles of barley grain. *Metabolomics*, 11, 454–467.
- Wishart, D. S., (2011). Advances in metabolite identification. *Bioanalysis*, 3, 1769–1782.
- Xu, J., Chen, Z., Wang, F., Jia, W., & Xu, Z., (2020). Combined transcriptomic and metabolomic analyses uncover rearranged gene expression and metabolite metabolism in tobacco during cold acclimation. *Sci. Rep.*, 10, 5242.
- Xu, S., Hu, C., Hussain, S., Tan, Q., Wu, S., & Sun, X., (2018). Metabolomics analysis reveals potential mechanisms of tolerance to excess molybdenum in soybean seedlings. *Ecotox. Environ. Saf.*, 164, 589–596.
- Yang, C., Yang, H., Xu, Q., Wang, Y., Sang, Z., & Yuan, H., (2020). Comparative metabolomics analysis of the response to cold stress of resistant and susceptible Tibetan hulless barley (*Hordeum distichon*). *Phytochemistry*, 174, 112346.
- Yang, M., Yang, J., Su, L., Sun, K., Li, D., Liu, Y., Wang, H., et al., (2019). Metabolic profile analysis and identification of key metabolites during rice seed germination under low-temperature stress. *Plant Sci.*, 289, 110282.
- Zhang, Q., & Dai, W., (2019). Plant response to salinity stress. In: Dai, W., (ed.), *Stress Physiology of Woody Plants* (pp. 155–173). CRC Press, Boca Raton.
- Zivy, M., Wienkoop, S., Renaut, J., Pinheiro, C., Goulas, E., & Carpentier, S., (2015). The quest for tolerant varieties: The importance of integrating “omics” techniques to phenotyping. *Front Plant Sci.*, 6, 448.
- Zoghi, Z., Hosseini, S. M., Kouchaksaraei, M. T., Kooch, Y., & Guidi, L., (2019). The effect of biochar amendment on the growth, morphology and physiology of *Quercus castaneifolia* seedlings under water-deficit stress. *Eur. J. For. Res.*, 138, 967–979.
- Zörb, C., Geilfus, C. M., Mühling, K. H., & Ludwig-Müller, J., (2013). The influence of salt stress on ABA and auxin concentrations in two maize cultivars differing in salt resistance. *J. Plant Physiol.*, 70, 220–224.

# Index

---

## 1

1,2-dichloroethane, 14  
1-aminocyclopropane-1-carboxylate, 19, 44, 58, 66  
1-pyrroline-5-carboxylate synthase, 423

## 2

2,4-dichlorophenoxyacetic acid (2,4-D), 24  
2-[4-carboxyphenyl]-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, 104  
2D-PAGE, 402, 414

## 3

3-methoxytyramine, 438

## 5

5-azacytidine, 268, 370  
5-methylcytosine (5mC), 361, 363  
specific antibody, 378

## 7

7-methylguanosine cap, 353

## $\alpha$

$\alpha$ -amylase, 107, 112, 142, 147, 148, 151, 152  
activity, 112  
 $\alpha$ -ketobutyrate, 73  
 $\alpha$ -ketoglutaric acid, 437  
 $\alpha$ -tocopherol, 104, 107, 155, 441

## $\beta$

$\beta$ -cyanoalanine synthase, 416

## $\gamma$

$\gamma$ -amino butyrate shunt, 415  
 $\gamma$ -aminobutyric acid, 437

## A

### Abiotic

environmental  
factors, 250  
stressors, 351  
stress, 41, 42, 48, 50, 51, 58, 60, 62, 64, 66, 67, 72–74, 77, 80–84, 99, 100, 103, 105, 107, 108, 114, 116, 134, 138, 147, 158, 167–169, 172, 173, 176–181, 187–195, 201–205, 209, 210, 239, 246, 250–252, 254, 266, 267, 279, 283, 284, 306, 307, 316, 318, 321, 330, 333, 334, 349–351, 353–355, 357, 365, 368, 372–375, 380–386, 401–404, 413–416, 420–425, 431–435, 442, 443  
mechanism, 401, 432  
tolerance, 67, 74, 81, 83, 103, 108, 172, 177, 179, 188, 190–192, 194, 202, 204, 205, 209, 267, 279, 401, 403, 432  
traits, 190  
variables, 42  
stressors, 42, 61, 66, 284, 368, 375, 380, 382, 384, 385, 415  
tensities, 42, 43  
environments, 43

Abscisic acid (ABA), 18, 44, 45, 63, 66, 68, 72, 73, 82, 115, 153, 154, 169, 171, 177, 194, 202, 244, 308, 357, 413  
dependent stomata closure, 63  
independent pathways, 202

### Abscission, 113

*Acaulospora longula*, 80

1-aminocyclopropane-1-carboxylate (ACC), 22, 23, 43, 44, 47–49, 58, 60, 66, 68, 69, 72–75, 77, 180  
deaminase, 22, 23, 44, 43, 47, 48, 60, 68, 69, 72–75, 77, 180

Acclimatization process, 59

Acetic acid, 21, 44, 440

Acetyl, 353, 373

- Acetylated histones, 374
- Acetylation, 352, 357, 359, 361, 367, 371, 372
- Achromobacter xylosoxidense*, 68
- Acquired systemic resistance (ASR), 41
- Acremonium*, 14, 65
  - typhinum*, 65
- Acrocalymma vagum*, 69
- Actinobacteria, 49
- Activation of,
  - antioxidant systems, 140, 155
  - ion channels, 252
  - membrane efflux, 108
- Activity-based protein profiling (ABPP), 404
- Adenine methylation, 370
- Adenylation, 257
- Adsorbent use, 6
- Adsorption/desorption of metal ions, 20
- Adverse environmental conditions, 59, 72, 80, 108, 113, 422
- Aerated hydration, 114
- Aeration, 10, 101
- Aerobic
  - bacteria, 13
  - biodegradation, 15
  - condition, 15
  - degradation, 14, 15
  - metabolisms, 153
- Affinity purification-based approaches, 403
- Agmatine, 308
- Agricultural
  - genetic improvement, 306
  - land, 79, 179, 308
  - pests, 136
  - production, 134, 173
  - productivity, 363, 367
  - sector, 61, 73, 157
  - sustainability, 134, 140, 157
- Agrobacterium-mediated transformation, 334
- Agronomic
  - management, 167
  - practices, 179
  - traits, 284
  - importance, 138
- Alcaligenes*, 13
- Aldrin, 6
- Alfalfa, 23, 110
- Algae biomass, 15
- Algae*, 15
- Alkalinity tolerant proteins, 51
- Allium*
  - cepa*, 82, 102, 144
  - porrum*, 109
- Alpha-ketobutyrate, 44
- Alteration of membrane integrity, 113
- Alternaria*
  - brassicicola*, 171
  - solani*, 86
- Alternating current, 156
- Alternative oxidase (AOX), 408, 415
- Aluminum, 363, 365, 441
- Alyssum bertolonii*, 21
- Arbuscular mycorrhizal fungi (AMF), 17, 46, 57–60, 76, 79–84, 86
  - colonization, 42–44, 46, 48, 49, 79, 82–86
  - induced resistance, 86
  - mediated plant growth, 80
  - colonized plant, 86
- Amine oxidase, 153, 373
- Amino acids, 45, 112, 144, 152, 208, 308, 352, 371, 376, 423, 433, 435, 437, 438, 440
  - metabolism, 414, 440
- Amplified fragment length polymorphism (AFLP), 174, 181, 315, 330
- Amylase, 105, 115, 141
- Anaerobic
  - bacteria, 13–15
  - degradation mechanisms, 15
  - functions, 13
  - microorganisms, 12
  - respiration, 16
- Anaerobically degradable polluted soil, 10
- Angiosperms, 65, 369
- Antagonism, 69
- Antagonistic
  - mechanism, 60, 76
  - properties, 60
- Anthocyanin, 309, 433
- Anthracene, 14
- Anthropogenic activities, 3, 4, 6, 30, 250, 355, 362, 440
- Anti-pathogenic metabolites production, 60
- Antibacterial activities, 18

Antibiotic resistance proteins, 20  
 Antifungal biochemicals, 72  
 Antimicrobial  
   activity, 69  
   molecules, 73  
 Antioxidant, 104, 113, 438  
   activities, 110, 146  
   defense, 111, 112, 115, 149, 416, 422  
   enzymes, 58, 63, 68–71, 74–76, 82,  
     84–86, 103, 104, 114, 136, 140, 142,  
     178, 179, 244, 245  
   alternation, 105  
   system, 68, 81  
   metabolites, 63  
   system, 68, 102, 155  
 Antioxidative  
   defense mechanism, 401, 419  
   enzymes, 100, 253  
 Antiparallel direction/singlet state, 152  
 Amine oxidase (AOs), 153  
 Apoplast, 403, 410, 418  
 Apoplastic spaces, 153  
 Apoptosis, 256  
 Aquaglycoporins, 365  
 Aquaporins (AQP), 70, 101, 111, 424  
 Aquatic  
   environment, 25  
   systems, 25  
 Arabidopsis, 44, 68, 70, 73, 115, 170–172,  
   176, 177, 179, 195–203, 205–207,  
   243–245, 252, 253, 257, 264, 267–269,  
   277–279, 285, 317, 333, 334, 337, 338,  
   356, 357, 359, 361, 363, 367, 369–373,  
   375, 381, 383, 385, 405, 406, 409, 411,  
   413–415, 417, 419, 439  
   H2AK121 monoubiquitination, 372  
   *A. thaliana*, 44, 68, 70, 73, 172, 176, 264,  
     267, 269, 279, 317, 333, 334, 337, 338,  
     363, 367, 373, 383, 385, 405, 406, 409,  
     411, 439  
   transgenic plants, 205  
 Arbuscular mycorrhiza (AM), 17, 41, 42,  
   46, 57, 59, 76, 79, 87  
   fungi, 17, 46, 57, 59  
   symbiosis, 316  
 Archaeal adaptive immune system, 332  
 Architectural alterations, 365  
 Arginine, 354, 373, 437, 440

  methyltransferases, 373  
 Argonaute (AGO), 251, 254–256, 258, 260,  
   262, 269, 274, 352, 353, 375, 276  
   mediated complexes, 352  
   proteins, 258, 375  
     family, 251  
 Arsenic, 21, 49, 69, 71, 335, 365  
   tolerant plants, 365  
 Artificial transcription factors, 190, 200, 208  
 Ascorbate peroxidase (APX), 20, 58, 63, 68,  
   70, 104, 105, 141, 143, 149, 155, 244, 245,  
   406, 407, 409, 410, 417, 418, 422, 425  
 Ascorbic acid, 45, 47, 101, 104, 107, 144,  
   155  
 Aspartate, 408, 416, 437  
   aminotransferase, 408, 416  
 Atropine, 433  
 Autochthonous, 4  
 Automated large-scale sequencing, 386  
 Automobiles, 111  
 Auxin, 44, 46, 66, 73, 82, 85, 102, 151, 275  
   signaling pathways, 102  
*Avena sativa*, 436  
*Azospirillum*, 42, 44–47, 60, 67, 75, 77  
   *brasilense*, 44, 75  
*Azotobacter*, 19, 42, 60, 77

## B

*Bacillus*, 14, 19, 22, 42, 45, 47, 48, 60, 66,  
   68, 70, 71, 73, 76, 77, 175  
   *amyloliquefaciens*, 45, 47, 76  
   *cereus*, 68  
   *pumilus*, 48  
   *subtilis*, 48, 68, 70, 73  
   *thuringiensis*, 175  
 Bacterial endophytes, 59, 65, 66  
 Barley genotypes, 245  
 Basic  
   helix-loop-helix (bHLH), 270, 270, 406,  
     413  
   leucine zipper (bZIP), 188, 194, 197, 203,  
     204, 206, 406, 413, 436  
 Begomoviruses, 337  
 Beneficial microorganisms, 42, 79  
 Benzopyrene, 14  
 Betaine aldehyde dehydrogenase 1  
   (BADH1), 321  
 Bioaccumulation of microbes, 49

- Bioaugmentation, 10
- Biochar, 18, 19
- Biochemical, 25, 41, 43, 50, 62, 66, 72, 75, 82, 86, 100, 101, 107, 108, 112–115, 134, 135, 142, 147, 151, 157, 187, 188, 191, 201, 240, 244, 277, 279, 283, 310, 350, 380, 432, 437, 441
  - cell repair systems, 101
  - changes, 50, 62, 100, 244, 432
  - pathways, 134, 188, 283
- Biochemistry, 42
- Biocontrol activity, 76
- Biodegradable waste, 6
- Biodegradation, 9, 15, 23
- Bioengineering plants, 209
- Biofilms, 18
  - formation, 46
- Biofuel production, 15
- Biogenesis, 251, 253–256, 258, 260, 262, 265, 277, 278, 283, 414
- Biohazardous contaminants, 4
- Bioinformatics, 191, 192, 240, 243, 267, 280–282, 284, 322
  - approaches, 306, 403
  - science, 190
  - techniques, 378
- Biological
  - management, 50
  - pathways, 322, 424
  - pollutants, 7
  - system, 5, 13, 151, 152, 195, 280
- Biomass, 18, 24, 45–47, 64, 67, 71, 73, 75, 79, 80, 83–85, 100–102, 104, 106, 107, 114, 116, 141, 142, 145, 146, 148, 149, 188, 433, 435, 441
  - accumulation, 83, 106, 107, 142, 188
  - production, 75, 83, 101, 146, 149
  - yield, 64, 67, 83
- Biomolecules, 103, 106, 262, 433
- Biomonitor species, 25
- Bioremediation, 3, 4, 5, 6, 7, 9, 11, 13–15, 17, 18, 20, 25, 29, 30
  - contaminated sites, 4
  - process, 9, 15, 29
  - technique, 7, 29
  - technology, 4
- Biore Restoration, 5
- Biosorb organic, 14
- Biosorption, 15, 21
- Biosparging method, 10
- Biosurfactants, 24
- Biotechnological
  - approaches, 181, 250, 252
  - efforts, 365
- Biotechnology, 12, 181, 188, 240
- Biotic stress, 20, 41, 42, 50, 51, 57, 58, 62, 63, 69, 147, 156, 167, 171, 172, 174, 175, 180, 181, 265, 307, 317, 320, 350, 380, 432–434
- Biotinylation, 352
- Biotransformation, 16, 20
- Biotreatment, 5
- Bioventing, 10
- Biowaste treatment, 12
- Bisulfite
  - conversion, 379
  - sequencing, 379
- Blight enterobacterial pathogen, 337
- Blooming, 42, 365
- Blue light-dependent anthocyanin, 145
- Blue native-polyacrylamide gel electrophoresis, 415
- Blumeria graminis* f. sp.
  - Graminis, 171
  - tritici* (Bgt), 337
- Boron, 440
- Botrytis* sp., 76
- Brassica*
  - campestris*, 359
  - chinensis* L., 21
  - juncea*, 71, 78, 318, 366
  - napus*, 21, 317, 337, 364
  - rapa*, 143, 379
- Breeding
  - populations, 191
  - programs, 181, 320, 339
  - schemes, 190
- Broad spectrum
  - biofertilizer, 79
  - RNA silencing, 265
- Bryophytes, 257
- Burkholderia*, 23, 24, 42, 65, 70, 77
  - kukruiensis*, 65
  - phytofirmans* PsJN, 24
- Burkholderiaceae*, 20

## C

- Cadmium, 21, 49, 69, 106, 111, 136, 156, 196, 198, 335, 336, 441
  - resistant endophytic bacterium, 21
- Caenorhabditis elegans*, 28, 265
- Calcineurin B-like (CBL), 360
  - protein 10 (CBL10), 356
- Calcium, 47, 58, 67, 169, 309, 363, 364, 440
  - dependent protein kinase, 58, 364
  - ions, 169
- Calnexin, 411, 419, 422
- Calreticulin, 422
- Cambium differentiation, 105, 152
- Camellia sinensis*, 360
- Candidate gene information, 319
- Cannabis sativa* L., 363
- Canola, 311, 441
- Capillary
  - electrophoresis mass spectrometry, 434
  - sequencing, 314
- Capsicum annuum*, 77, 142, 170
- Carbon, 14, 15, 24, 29, 79, 146, 309, 319, 366, 369, 416, 440
  - assimilation mechanism, 309
  - dioxide, 15, 319, 366
- Carbonic anhydrase, 102, 107, 149, 409
- Carotenoids, 63, 105, 149, 155, 433, 437
- Catabolic
  - pathway, 20
  - reaction, 13
- Catalase, 18, 20, 45, 58, 63, 68, 70, 76, 101, 103, 105, 108, 114, 115, 136, 141, 149, 155, 178, 244, 245, 409, 422
- Catalytic destruction, 6
- Catharranthus roseus*, 74
- Cell
  - cycle-related processes, 114
  - differentiation, 151, 256
  - division, 16, 102, 108, 151, 358
  - elongation, 108, 151, 357, 418
  - encapsulation, 10
  - membrane
    - permeability, 112
    - rupture, 16
    - stability, 111
    - thermostability, 170
    - ultrastructure, 151
  - organelle, 412, 420, 424, 425, 442
  - wall, 410
    - degrading lytic enzymes, 76
    - lignification, 419
    - modification, 410, 418
    - protein, 405, 418
- Cellular
  - activities, 317, 356, 367, 374, 375, 420
  - compartments, 192, 424
  - defensive mechanisms, 417
  - dehydration, 62
  - fractionation, 403
  - homeostasis, 266, 402
  - localization, 403
  - machinery, 103, 194
  - membrane integrity, 105
  - organelles, 404, 424
  - organization, 62
  - permeability, 151
  - processes, 194, 259, 267, 402
- Cellulases, 65
- Central regulatory proteins, 195
- Centromeres, 283
- Chaperones, 170, 406, 413, 422
  - proteins, 420
- Charcoal root rot disease, 86
- Chelating agents, 24
- Chelation, 21
- Chemical
  - approaches, 135
  - fertilizer, 69
  - priming, 144
  - technologies, 20
- Chickpea, 68, 102, 103, 109, 111, 113, 138, 145, 146, 413, 418
- Chilling, 59, 68, 70, 106, 112, 113, 149, 336
  - conditions, 59
- Chinese spring, 408, 416
- Chitinase, 43, 60, 72, 176, 406, 418
- Chloride, 179, 356
- Chlorinated pesticides, 13
- Chlorine, 46, 49, 440
- Chlorophyll
  - chlorophyll-a, 77, 145
  - degradation, 441
- Chloroplasts, 147, 153, 240, 403, 404, 416, 422
  - membrane proteins, 417
- Chlorosis, 364

- Chromatin, 251, 254, 258, 264, 268, 350, 352, 353, 357–359, 361, 368–381, 385, 386, 413, 414  
   immunoprecipitation, 377  
   modification ATPase, 380  
   remodeling, 254, 350, 359, 361, 375, 379, 380, 385, 406, 413, 414  
 Chromatographic separation techniques, 434  
 Chromium, 321, 322, 336, 441  
 Chromodomain, 372, 380  
 Chromomethylase 3, 370  
 Chrysanthemum morifolium plants, 177  
*Cicer arietinum*, 45, 102, 138, 146, 148, 406, 407, 410, 437  
 Cis-acting siRNAs, 258  
 Citric, 21, 414, 439, 441  
*Citrullus lanatus*, 177  
 Clavicipitaceous endophytes, 65  
 Climate-smart crops, 339  
 Clinical diagnoses, 242  
 Coat protein, 177, 337  
 Cobalt, 440  
 Cold  
   acclimation, 359, 419, 439  
   induced genes, 359  
   responsive, 359  
   shock-domain family proteins, 193  
   stress acclimatization, 417  
 Commercial  
   crop cultivars, 189  
   exploitation, 203  
   field applications, 6  
   manipulation, 188  
   remediation processes, 29  
   tetraploid, 191  
 Communication devices, 135  
 Comparative genomic approach, 267  
 Compartmentalization, 438, 442  
 Compatible  
   quantum incitement, 152  
   solutes, 63, 103, 192, 193, 443  
 Complementary sequences, 258  
 Complex  
   chemical substances, 24  
   modular structure, 332  
   multicomponent signally pathways, 187  
   multigene nature, 189  
   regulatory circuits, 201  
   stress-responsive regulatory pathways, 188  
   traits, 203, 307, 310, 329  
 Comprehensive genome-wide analysis, 204  
 Computational  
   biology, 279  
   techniques, 279  
 Contaminated  
   food crops, 365  
   materials, 12  
   soil  
     bioremediation, 17  
     decontamination, 24  
 Contemporary breeding methods, 250  
 Conventional  
   breeding, 189, 190, 330, 339  
   efforts, 190  
   methods, 5, 29  
   physical assessment, 25  
   plant breeding, 168, 190  
   seed priming methods, 133  
 Copper, 21, 49, 79, 272, 276, 336, 361, 366, 410, 440  
   resistant endophytic bacteria, 21  
   zinc superoxide dismutase, 272, 361  
 Cost-effective  
   method, 158  
   seed enhancement technique, 137  
 Cotton leaf curl disease, 172  
   Kokhran virus, 337  
 Cowpea trypsin inhibitor, 175, 181  
*Craterostigma plantagineum*, 278  
 C-repeat-binding factors, 205, 336, 359  
 Clustered regularly interspaced short palindromic repeat (CRISPR), 176, 188, 201, 208, 210, 329–339  
   associated protein, 176, 201, 329–334, 337, 339  
 Cas, 329–339  
   Cas9, 188, 208, 210  
     mediated genome editing, 329  
   CasiRNAs, 258  
   endonuclease, 330–332, 339  
   RNA, 332  
*Cronobacter dublinensis*, 73  
 Crop  
   adaptation, 246  
   development, 385  
   establishment, 100, 101, 103, 133

- fecundity, 42
  - growth, 134, 140, 146, 158
  - improvement, 250, 278, 283, 306, 313, 323, 329, 331, 333, 339, 432
  - plant improvement, 207
  - production, 58, 251, 349, 356, 358, 365
  - productivity, 50, 75, 103, 106, 133, 157, 181, 308, 319, 322, 352, 358, 360, 368
  - Cr-stressed sunflower plants, 441
  - Crude oil contamination, 24
  - Cryptochrome circadian regulator 2, 145
  - Cryptococcus* sp. CBSB78, 21
  - Cryptotaenia japonica*, 138
  - Cucumber cotyledons, 107
  - Cucumber mosaic virus (CMV), 337
  - Cucumis sativus*, 85, 102, 140–143, 334, 338
  - Cucurbita maxima*, 79
  - Curvularia protuberata*, 69
  - Cyclin dependent protein kinase (CDK), 58
  - Cyclotron resonance model, 152
  - Cytochrome-c oxidase subunit 6b, 408, 415
  - Cytokinin, 44, 46, 72, 73, 75, 77
  - Cytosegresomes, 144
  - Cytosine methylation, 208, 356, 369, 370
  - Cytoskeleton structure, 62, 309
  - Cytotoxicity, 356, 362
- D**
- D-alanyl-D-serine, 438
  - DCas9-mediated multiplexing, 209
  - De novo*
    - methylation, 258, 370
    - synthesis, 151, 201, 369
  - Deacetylation, 357, 358
  - Dechlorination, 14
  - Decomposition routes, 26
  - Defense mechanism, 100, 101, 108, 110, 168, 194, 363
  - Defensive reaction mechanisms, 50
  - Degradation
    - kinetics, 30
    - organic substances, 10
  - Dehydration
    - cycle, 100, 101
    - period, 413
    - responsive
      - adaptation, 413
      - element binding protein 2A (DREB2A), 360
      - stress-response, 381
  - Dehydrins, 101
  - Dehydroascorbate reductase, 244
  - Dehydroascorbic acid, 104
  - Dehydrogenase, 152, 409, 417
  - Demeter-like 2 (DML2), 370
  - Demethylation, 16, 361, 370, 373
  - Denitrifiers, 15
  - Deoxynivalenol, 76
  - Dependent biotin identification, 404
  - Dermatitis, 441
  - Desorption electrospray ionization mass spectrometry, 435
  - Destabilization of primary transcript, 264
  - Detoxification, 7, 9, 11, 14, 16, 24, 26, 28, 49, 104, 318, 321, 417, 418
  - Deubiquitinases, 371
  - Developmental
    - plasticity, 283
    - processes, 151, 249, 257, 258, 277, 320
    - stages, 107, 172
  - Diacylglycerols, 201
  - Dicer like 1 (DCL1), 255, 258, 260, 273–275, 277
  - Dicer-mediated cleavage, 262
  - Dieldrin, 6
  - Differential display PCR, 241
  - Differentially expressed genes, 319, 322
  - Diphenylene iodonium, 104
  - Direct
    - causing organisms, 176
    - cloning, 280
    - current, 156
    - defiance, 43
    - flow injection mass spectrometry, 431
    - infusion mass spectrometry, 434
    - progression, 63, 76, 86
    - resistance, 50, 171, 176, 311, 322
    - vulnerability, 51
  - Distress-tolerating microorganisms, 43
  - Diversity arrays technology, 330
  - DNA
    - binding
      - domains, 194, 200
      - properties, 209
    - fragments, 12, 314, 377



- markers, 174
  - methylation, 258, 262, 268, 278, 282, 351, 352, 355–357, 359, 361–363, 368–372, 375, 376, 378–380, 382, 385, 386
    - profiles, 361, 376
  - methyltransferases, 353, 369
  - microarray, 240, 243, 377, 378
  - molecular markers, 312
  - processing, 108
  - protein
    - associations, 380
    - interaction, 376
  - related alterations, 380
  - repair machinery, 114
  - replication, 352, 369
  - sequencing techniques, 191
  - technology, 12, 168, 330
  - Domains rearranged methylase, 370
  - Domestic waste, 7
  - Dormancy
    - breakdown, 154
    - breaking, 153
  - Dose-dependent hypermethylation, 363
  - Double
    - membrane enveloped organelles, 403
    - strand break, 329–333
    - stranded
      - DNA material, 314
      - DsRNA, 251, 254, 255, 258, 260, 262, 265, 266, 277, 278, 353
  - Downstream stress-responsive genes, 205, 209
  - Drought, 42, 44, 45, 48, 49, 51, 58–63, 67–70, 72–75, 80–82, 100, 103, 105, 107, 110, 111, 145, 147, 156, 167, 168, 170, 174, 177–181, 187–191, 193, 196–200, 202, 204–207, 240, 241, 244, 251, 307, 308, 316–319, 333–336, 350, 355, 357, 358, 366, 368, 373, 375, 381, 382, 384, 413, 414, 416–418, 423, 433–437, 442
    - frequency, 435
    - inducible downstream genes, 206
    - mitigation, 81
    - resistance, 49, 73–75, 111, 316
    - sensitive, 318, 319
    - stress, 45, 48, 81, 111, 180, 188, 244, 357, 358, 436
    - tensile tolerance, 46
    - tolerance, 68, 73, 74, 81, 82, 105, 147, 191, 196, 197, 205, 207, 244, 317, 333, 334, 384, 418, 436
    - tolerant, 170, 318, 319
  - Drug discovery, 242
  - Drum priming, 109
  - Dry
    - seed priming treatment, 134
    - weight under saline conditions, 104
  - Dynamic
    - mechanism, 252
    - reprogramming of transcription, 349
  - Dynamo motion, 137
- ## E
- Earias vittella*, 175
  - Early
    - reverse mobilization, 108
    - seedling developmental stages, 140
  - Ecology enhancement, 20
  - Economic products, 168
  - Economical development of farmers, 139
  - Ectomycorrhizae, 79
  - Efficient mixed-model association, 311
  - Eggplant, 76, 144, 175
  - Electrical
    - conductivity, 143, 179
    - signaling, 137
  - Electromagnetic field, 134, 135, 139, 140, 145, 149, 156, 158
  - Electromagnets, 135, 139
  - Electron
    - acceptors, 9, 104
    - transfer chain, 102, 309, 414, 415
  - Electronic
    - configuration, 134
    - wastes, 4
  - Electrophilic endobiotics, 28
  - Electrostatic interaction, 15
  - Elevated systemic toleration, 44
  - Endochitinase gene, 172, 176
  - Endogenous, 208, 258, 260, 265, 277, 278, 282, 331, 334
    - promoter, 208
  - Endogenous
    - siRNAs, 258
    - mediated regulations, 277

- sRNAs, 251
- Endohydrolase, 151
- Endonucleases, 254, 258, 332
- Endophyte, 22, 23, 66, 67
  - colonization, 24
  - genera, 20
  - assisted phytoremediation, 21
  - infected plants, 24
- Endophytes, 20–22, 57–59, 64–72, 75, 79, 86, 87
  - interaction, 59, 64, 66
  - mediated stress tolerance, 59
- Endophytic
  - bacteria, 20, 24
  - fungus, 24
  - infection, 67
  - microbes, 65
  - microorganisms, 46, 64, 67, 69
  - organisms, 64, 65
  - yeasts, 21
- endoplasmic reticulum (ER), 403, 420, 422, 423, 425
- Endo-proteases, 152
- Endosperm weakening, 108
- Endrin, 6
- Energy
  - consumption, 10
  - transformation, 134
  - utilization, 309
- Engineered
  - ascorbate peroxidase, 404
  - bioremediation, 9
  - in situ* bioremediation, 9
- Enhanced
  - glycolytic pathways, 440
  - heat tolerance, 206
  - nutrients uptake, 73
  - soil quality, 79
- Enterobacter*, 19, 23, 42, 49, 60, 65, 75
  - asburiae*, 65
- Enterobacteriaceae*, 20
- Environmental
  - biomonitoring, 25
  - constraints, 189, 192, 285
  - contamination, 41
  - distresses, 240
  - extremes, 306
  - extremities, 136, 146
  - fluctuations, 432
  - pollutants, 4, 12, 30
  - pollution, 3, 13, 57
  - pressures, 306
  - stimuli-caused signals, 201
  - stress, 25, 26, 42, 59, 61, 63, 64, 108, 113, 136, 156, 190, 192, 202, 207, 209, 240–243, 245, 285, 305–307, 310, 319–321, 323, 329, 339, 355, 356, 382, 431, 433–436
  - stressors, 59, 356, 367
  - sustainability, 20
- Environment-friendly agro-technique, 157
- Environs reliability, 42
- Enzymatic
  - activities, 110
  - activity, 138, 258, 260, 262, 438
  - antioxidants, 244, 245
  - pathway, 13
  - reaction, 62
  - substitution, 369
- Enzyme degradation, 108
- Epicotyl cells, 144
- Epigenetics, 303, 349–351, 385, 386
  - alterations, 357, 363, 369, 382
  - changes, 349–351, 361, 368, 372, 376, 380, 381
  - inscriptions, 363
  - machinery reprogramming, 386
  - markers, 371
  - mechanism, 351, 363, 370
  - memory, 380
  - modifications, 350, 352, 386
  - processes, 350–352
  - profile, 377
  - regulation, 352, 358, 372, 380, 381
  - regulators, 372–374
- Epigenome-related methods, 377
- Erwinia amylovora*, 176, 337
- Escherichia*, 14
- Essential elements, 362
- Ethanolamine, 438
- Ethylbenzene, 11
- Ethylene, 44–49, 51, 58, 60, 63, 66, 68–70, 72–75, 77, 195, 271, 274, 333, 367
  - production, 43
  - response factors VII (*ERFVII*s), 367
  - synthesis, 48

Eukaryotic  
   genomes, 283  
   organisms, 251, 254  
 Evapotranspiration, 20, 23, 24, 435  
   volatile contaminants, 20  
 Eventual pollutant destruction, 6  
 Excavation, 7  
*Exophiala pisciphila*, 69  
 Exopolysaccharide (EPS), 16, 18, 44, 48, 49  
   formation, 43  
 Expressed sequence tags, 280  
 Expression sequence tags (ESTs), 244, 246  
 External environmental factors, 25  
 Extracellular  
   matrix, 403, 418  
   metal sequestration, 15  
   polymeric molecules, 18  
 Extrusion, 16, 169

## F

Fatty acids desaturation, 171  
 Feedback regulatory mechanism, 277  
 Fermentor, 12  
 Ferredoxin-NADP<sup>+</sup> oxidoreductase, 409, 417  
 Ferric reductase activity, 106  
 Ferrimagnetism hypothesis, 153  
 Fertilizers, 4, 8, 41, 111  
 Feruloylagmatine, 438  
*Festuca arundinacea*, 23, 417  
 Firmicutes, 49  
 First generation sequencing, 314  
 Flavonoids, 63, 112, 433, 438, 441  
   biosynthesis, 440  
 Flavonol  
   3-O-galactoside, 438  
   biosynthesis, 440  
 Flooding tolerance, 82  
 Flora distribution, 50  
 Floral tissues, 175  
 Flow field-flow fractionation, 404  
 Food  
   FAO, 366  
   security, 62, 87, 134, 140, 157, 168, 339, 360  
 Foreign genomes, 331  
 Forward genetics, 280

Fourier transform ion cyclotron resonance  
   mass spectrometry, 434  
*Francisella*, 332, 333, 337  
   *novicida*, 333, 337  
 Free  
   amino acid synthesis, 46  
   enzyme bioremediation, 11  
   fatty acid, 436  
   flow electrophoresis, 404  
   living bacteria, 60  
   radical reactions, 152  
 Functional endonuclease protein, 256  
 Fungal  
   communities, 14  
   endophytes, 21, 59, 65, 67, 69, 72  
   feeders, 26  
   hyphae, 60  
   microorganisms, 60  
   pathogens, 69, 76, 334  
   symbionts, 79, 80, 83

Fungi, 19  
*Funnelformis mosseae*, 80, 82, 85, 86  
*Fusarium*, 14, 72, 76, 86, 176, 317  
   *gaminearum*, 76  
   *oxysporum*, 72, 86, 176

## G

Galactinol synthase, 170, 359  
 Gas chromatography-mass spectrometry, 431, 434  
 Gaseous exchange, 81, 82, 106, 366, 437  
 Gene  
   activity modulation, 362  
   expression, 50, 68, 76, 113, 176, 189, 191, 207, 208, 239–242, 245, 250, 251, 253–256, 258, 263, 264, 266, 268, 278, 280, 283–285, 307, 320, 322, 350–353, 356, 368, 370, 372–379, 385, 403, 413, 419, 420, 433  
   metabolites, 267  
   modification, 251  
   network, 106, 200, 241, 244, 245, 283, 320  
   regulation, 42, 194, 195, 205, 253, 256–259, 262, 263, 265–267, 277–280, 282, 371, 374, 380, 407, 413  
   regulatory networks, 187, 245  
   sequences, 320

- silencing, 251, 255, 258, 264–266, 268, 278, 279, 282, 332, 352, 379
- splicing, 253
- transmission, 173
- Generator stations, 111
- Genes of interest (GOI), 174, 330
- Genetic
  - approaches, 169
  - changes, 432
  - circuits, 194, 200
  - database resources, 306
  - disorders, 245
  - engineering, 75, 168, 187, 189–191, 194, 195, 202, 203, 240, 245, 252, 330, 339
  - epigenetic variations, 379
  - improvement programs, 365
  - manipulation, 193
  - mechanism, 168
  - modification, 20, 29
  - network, 194, 242
  - variants, 306, 307, 316, 319
- Genetically
  - improved crops, 323
  - modified, 30, 168, 203, 330
  - destabilization, 364
- Genome
  - editing, 190, 200, 210, 329, 330, 332–334, 337, 339
  - engineering, 339
  - phenotype association studies, 307
  - plants, 256
  - sequences, 280, 313, 380
  - stability, 251
  - survey sequences, 280
  - wide
    - association studies (GWAS), 305–307, 310–323
    - marker selection tool, 319
    - identification, 209, 250
    - profiling, 377
    - sequencing, 434
- Genomic, 168, 189, 191, 207, 255, 280, 284, 305, 306, 314, 320, 330, 332, 351, 352, 375, 376, 378, 379, 384, 402
  - database, 379
  - sequence, 306, 351
  - stability, 255, 375
  - phenotype
    - affiliations, 312
    - mapping, 283
    - relationships, 306
- Genotypes, 167, 305, 312–316, 318, 365, 383
- Genotypic
  - information, 313
  - qualities, 312
- Genotyping, 312, 313
- Gentisic acid, 438
- Geological time scale analysis, 59
- Geomagnetic, 106, 134, 135, 137, 138, 144, 151, 156, 157
  - field, 106, 134, 135, 137, 138, 144, 151, 156, 157
- Geo-magnetotropic, 135
- Germination, 77, 103, 107, 141, 142
  - magnetic constant, 138
  - parameters, 139
  - percentage, 103, 112, 138–140
  - seedlings, 61, 101
  - tolerance index, 103
- Germinative metabolic activities, 100
- Germin-like proteins, 418
- Germline cells, 259
- Germplasm, 174, 315
- Gibberellic acid, 66, 147, 153, 154, 274
- Gigaspora*, 69
- Glechoma longituba*, 362
- Global
  - food
    - demands, 188
    - insecurity, 188
  - population, 134, 306, 367
  - temperatures, 360
  - warming, 62, 330, 339, 360, 366
- Globular histone proteins, 352
- Glomalin, 79, 87
- Glomeromycota, 79
- Glomus* sp., 83
- Glucanase, 43, 172, 176, 406
- Gluconacetobacter diazotrophicus*, 65
- Gluconate, 439
- Glucose metabolism, 418
- Glucosinolates, 441
- Glutamate, 409, 417, 437, 438
- Glutathione, 20, 24, 26, 45, 63, 101, 111, 112, 114, 116, 155, 178, 244, 246, 422, 441

peroxidase, 45, 155, 244–246, 406, 407, 422  
 reductase, 63, 101, 114, 116, 155, 178  
 Glutathione-S-transferase, 24  
 Glyceric acid, 437  
*Glycine*, 63, 66, 70, 82, 168, 180, 193, 407, 414, 423, 437, 438  
 betaine, 63, 70, 82, 168, 180, 193, 423, 438  
*max*, 22, 85, 86, 102, 139, 141, 142, 148, 149, 179, 180, 264, 270, 333, 334, 407–411, 437  
 Glycophytes, 252, 285, 356  
 Glycophytic plants, 356  
 Glycoprotein glomalin, 79  
 Glycoside hydrolase, 408, 415  
 Glycosylation, 371  
*Gossypium raimondii*, 264, 271  
 G-protein  
   coupled receptors, 201  
   signaling, 418  
 Gravitropism, 137  
 Groundwater contamination, 3  
 Growth retardation, 112, 206  
 Gymnosperms, 65  
*Gymnosporangium juniper-Virginiana*, 176

## H

Hairpin-like secondary structure, 280  
 Halophyte, 285, 356, 438  
 Hazardous  
   chemicals, 26, 28, 156, 350  
   metal bioavailability, 28  
 Heat  
   induced miRNAs, 361  
   shock  
     elements' binding site, 170  
     factor genes, 179  
     protein, 25, 108, 112, 113, 170, 179, 193, 334, 360, 361, 364, 381, 415, 422  
     transcription factor A1s, 360  
   stress, 25, 58, 62, 170, 171, 178, 179, 181, 193, 206, 309, 318, 321, 358, 360, 361, 376, 382, 439, 440  
   response, 321, 360  
   rice plants, 439  
 Heavy metal, 3–5, 9–11, 14–22, 25, 26, 28–30, 42, 49, 51, 58–62, 69, 72, 74–76, 80, 81, 83, 85, 103, 106, 111, 112, 156, 167, 241, 307, 321, 350, 355, 362–364, 369, 382, 416, 433, 440–442  
   absorption investigations, 26  
   administrations, 363  
   chelation, 81  
   compounds, 4  
   contamination, 69, 75, 76, 83  
   polluted soil, 17, 18  
   remediation, 18, 83  
   stress, 17, 42, 51, 58, 60, 62, 72, 74, 80, 83, 106, 112, 369, 382, 433, 441, 442  
   tolerant microorganisms, 49  
   toxicity, 75, 83, 103, 382  
   transporter P-type ATPases, 382  
*Helianthus annuus*, 102, 141, 144  
*Heliopsis virescens*, 175  
 Heliotropic, 135  
 Hemicellulases, 65  
 Hemichrome, 104, 107, 148  
 Heptachlor, 6  
 Herbicide resistance, 203  
 Herbivorous  
   animals, 307  
   insects, 86  
 Heterochromatic  
   histone H3 methylation, 372  
   siRNA, 254, 258, 260, 268, 277, 278  
 Heterochromatin, 251, 260  
 Heteromeric multisubunit effector  
   complexes, 331  
 Heterozygosity, 312  
 Hexachlorobenzene, 6, 8  
 Hexaphosphate, 201  
 High  
   abundant cytosolic proteins, 403  
   affinity K<sup>+</sup> transporters 1, 356  
   performance thin layer chromatography (HPTLC), 434  
   quality plasma membrane separation approach, 404  
   resolution genomic map, 378  
   resolution mapping, 310, 315  
   temperature reduced seed germination, 62  
   throughput  
     genomic techniques, 192

- methods, 241
    - microarray tool, 203
    - sequencing, 277, 279
    - tools, 189, 190, 201, 209
  - water permeability, 424
  - Histamine, 112
  - Histidine kinases, 201
  - Histone
    - acetylation, 359, 371, 373, 374
    - acetyltransferase (HATs), 359
    - alterations, 370
    - chaperones, 380
    - demethylases (HDMs), 357, 359, 371, 373, 374
    - hyperacetylation, 374
    - lysine methyltransferases (HKMTs), 373
    - methylation, 371, 373
    - methyltransferase (HMT), 373
    - modification, 350–354, 357, 358, 363, 371, 377, 382, 386
    - octamer, 352
      - H2A, 352, 353, 371, 372, 414
      - H2B monoubiquitination, 372
      - H3, 352, 353, 357, 361, 367, 371, 373, 383, 406, 407, 414
      - H3 lysine tri-methylation (H3K27me3), 357
      - H3K27me3, 357, 359, 371–373, 381, 383
      - H3Ser-10 phosphorylation, 357
      - H4, 352, 353, 357, 371, 373
    - proteins, 278, 352, 371, 376
      - electrostatic properties, 371
  - Homologous
    - recombination, 331
    - transcripts, 266
  - Homology
    - coordinated repair (HDR), 329, 331, 332, 336
    - directed repair, 332
    - related gene silence, 268
  - Hordeum*
    - distichon*, 440
    - vulgare*, 47, 83, 85, 102, 111, 142, 264, 335, 336, 359, 405, 436
  - Hormone
    - physiology, 48
    - regulation, 103
    - signaling, 111, 367
  - Horticultural crops, 168, 175
  - Host-induced gene silencing, 255
  - HUA ENHANCER1 (HEN1), 260
  - Human-produced contamination, 111
  - Hybridization, 172, 173, 181, 241, 243, 378
  - Hydathodes, 65
  - Hydraulic conductivity, 44
  - Hydro priming, 108, 111
  - Hydrocarbons, 5, 8
  - Hydrogen, 15, 26, 48, 63, 72, 104, 141, 147, 153, 169, 180, 422, 440
    - cyanide, 48, 72
    - peroxide, 26, 63, 104, 141, 147, 153, 422
  - Hydrolases, 418
  - Hydrolytic enzymes, 63, 141, 142, 151
  - Hydroprimed
    - maize, 113
    - plant material, 112
    - sunflower seeds, 110
  - Hydropriming, 99, 108–113, 115, 116
  - Hydroxyl radicals, 63, 422
  - Hyperaccumulation, 21, 438
  - Hyperaccumulator plant, 18
  - Hypermethylated loci, 364
  - Hyperosmotic stress, 419
  - Hypersensitive
    - reactions, 309
    - response, 63
  - Hyphal structures, 79
  - Hypoacetylation, 374
  - Hypocotyl length, 111
  - Hypomethylation, 363
  - Hyponastic leaves 1 (HYL1), 260
  - Hypoxia
    - reoxygenation reactions, 367
    - triggered gene expression, 366
  - Hyssopus officinalis* L., 105
- ## I
- Illumina/Solexa's sequencing technology, 320
  - Imitation switch, 380
  - Immunological memory, 101
  - In silico* methods, 266, 316
  - In situ*
    - bioremediation, 9
    - bioremediation processes, 9
  - Incineration, 5, 6

Indole acetic acid, 18, 19, 22, 23, 44, 47–49,  
66, 72, 73–77

#### Induced

membrane stability, 68  
systematic resistance, 50

#### Industrial

chemicals, 6  
equipment, 135  
pollution, 362

Industrialization, 3, 4, 69, 306, 362, 440

Inescapable geomagnetic field, 137

Inheritance, 251, 330, 372, 381, 385

#### Inhibition

antioxidants, 112  
cell wall extension, 365  
enzymes, 113

#### Inorganic

contaminants, 16, 29  
nutrients, 13  
pollutants, 4, 6, 7, 11, 14, 16, 22, 26, 29, 30  
salts, 99, 100

Insecticides, 136, 307

Intercellular connections, 375

International markets, 250

Inter-specific crosses, 173

Intracellular, 15, 20, 22, 144, 170, 201, 401,  
404, 420, 423

Intraspecific approaches, 173

Intrinsic *in situ* bioremediation, 9

Introggression of tolerance, 168

*In-vitro* tissue culturing, 333

#### Ion

exchange, 15, 21  
homeostasis, 169, 356, 423, 438  
toxicity, 62, 63, 67, 70, 73, 82, 109

#### Ionic

homeostasis, 103  
stress, 252  
substances, 144

Ionizing/nonionizing radiation, 99

Ionosphere, 137

Iron, 43, 72, 102, 106, 153, 200, 364, 440

Isoleucine, 308, 436, 438, 440

## J

Jasmonate, 51, 419

Jasmonic acid, 45, 51, 63, 66, 112, 309, 337,  
436

## K

*Klebsiella*, 42, 49, 60, 77

## L

Lachnospiraceae bacterium, 333

L-alanyl-L-glutamate, 438

Land farming, 11, 12

Larmor frequencies, 152

Laser capture microdissection (LCM), 403,  
404, 425

Late embryogenesis abundant, 101, 108,  
116, 170, 178, 193, 194

#### Lateral

differentiation of roots, 44  
root development, 365

Lead, 4, 11, 15, 49, 69, 115, 136, 145, 147,  
157, 168, 175, 188, 201, 206, 209, 243,  
250, 259, 267, 312, 321, 322, 329, 415,  
441, 442

#### Leaf

area index, 145  
chlorophyll content, 146, 156  
morphology, 67  
number, 141, 145  
orientation, 63, 113  
senescence, 75, 309

Leghemoglobin, 104, 148

Legumes, 79

Lenticels, 65

Lepidoptera, 175

*Leptotrichia wadei*, 337

Leucine, 195, 271, 273, 275, 276, 308, 361,  
364, 413, 438, 440

L-homomethionine, 438

Life technologies, 314

#### Light

absorbing flavoproteins, 153  
energy absorption, 136

Linkage disequilibrium, 310, 314, 315, 323

Linoleic acid, 436, 440

#### Lipid

composition, 63  
peroxidation, 20, 106, 110, 111, 141, 414,  
435

rich body areas, 28

Lipoxxygenases, 153, 418

Liquid  
  chromatography-mass spectrometry, 403,  
  407, 408, 411, 419, 431, 434, 440  
  crystal assembly, 152  
Living microbial biomass, 49  
L-malic acid, 439  
L-methionine, 438  
Long  
  noncoding RNA-mediated, 373  
  stranded RNA, 254  
Long-term  
  agricultural  
  development methods, 41  
  production, 42  
  sustainability, 29  
Low  
  cost seed enhancement technique, 99  
  frequency magnetic fields, 102  
*Lupine angustifolius*, 113  
*Lycopersicon esculentum*, 68, 84, 271  
Lysine  
  catabolism, 283  
  specific demethylase 1, 373

## M

Macromolecular interactions, 62  
Macromolecules, 147, 192, 404  
*Macrophomina phaseolina*, 86  
*Magnaporthe grisea*, 171  
Magnesium, 67, 79, 363, 440  
Magnetic  
  energy, 152  
  equator, 137  
  field, 81–83, 101, 102, 104–106,  
  134–147, 151–157  
  stimulus, 136, 140  
  treatment, 102, 151, 153  
Magnetized water, 158  
Magneto sensing mechanism, 152  
Magnetoprimed  
  induced tolerance, 104  
  wheat seeds, 104  
Magneto-priming, 100, 102, 103, 105, 106,  
  115, 116, 134, 137–140, 144, 147, 155,  
  156, 158  
  induced enhancement, 134  
Magnetosphere, 137  
Maize  
  plant salinity tolerance, 46

  seedlings, 318, 367  
Malate dehydrogenase, 416  
Malic acid, 440  
Malondialdehyde, 45, 77, 103, 107, 147,  
  359  
Maltose, 439  
*Malus domestica* Borkh., 337, 338  
Manganese, 49, 440  
*Manihot esculenta*, 335, 337  
Mannitol, 405, 423, 436, 438  
Marker-assisted  
  breeding, 181  
  recurrent selection, 174  
  selection, 174  
Marker-trait associations, 317  
Mass  
  accuracy instruments, 402  
  spectrometric approach, 419  
  spectrometry, 435, 443  
*Massilia* sp. RK4, 46  
Matrix-assisted laser desorption ionization,  
  403, 406–411, 413, 416, 419, 435  
Maturation, 42–44, 46, 49, 51, 63, 267, 370  
Mediated systemic resistance (MSR), 41  
*Medicago truncatula*, 112, 264, 271, 364  
Medical  
  equipment, 135  
  instruments, 157  
Melanin-like polymers, 21  
*Meloidogyne incognita*, 86, 175  
Membrane  
  composition, 113  
  conformation, 113  
  integrity, 139  
  lipids, 113  
  protection, 419  
Memory genes, 358, 381, 382  
Mercury, 364  
Meristematic tissue, 102  
Mesophyll tissues, 147  
*Mesorhizobium ciceri*, 68  
Metabolic, 16, 21, 29, 30, 100, 104, 105,  
  108, 110, 111, 114, 135, 144, 151, 153,  
  169, 189, 253, 268, 307, 317, 318, 320,  
  352, 356, 366, 403, 417, 431–434,  
  436–440, 442  
  activities, 105, 135  
  composition, 437



- engineering, 192, 193
- pathway, 151, 169, 317, 318, 320, 352, 431, 434, 436, 440, 442
- processes, 16, 111, 356
- reactions, 153
- repair, 108
- reprogramming, 439
- Metabolism, 12, 59, 62, 75, 79, 99, 100, 102, 108, 142, 146, 147, 175, 178, 283, 285, 307, 309, 316, 317, 350, 355, 357, 359, 365, 386, 403, 414, 416, 418, 420, 435, 436, 439, 440
- Metabolite production, 100, 104
- Metabolization, 28
- Metabolome, 110, 431–442
  - profile, 433–435, 437, 438
  - platform, 383
- Metabolomics, 385, 431–436, 442, 443
  - analysis, 434
  - database, 385
- Metal
  - binding ability, 83
  - biosorption, 21
  - chelation, 76
  - efflux pumps, 15
  - exclusion, 15
  - ion binding function, 102
  - ligand degradation, 15
  - organic complexation, 15
  - smelters, 111
  - transporter, 83, 321, 322
  - synthesis, 16
- Metallothioneins, 28
- Methane monooxygenase, 14
- Methanogens, 15
- Methionine, 438
- Methionine derivatives, 438
- Methyl, 104, 353, 369, 371, 373, 375, 423
- Methylation, 15, 16, 20, 259, 268, 282, 351, 352, 356, 359, 361, 363, 367, 369–373, 375, 378, 379, 382, 418
  - cytosine, 370
  - spread mechanism, 352
  - variation, 379
- Methyl-lysine-binding motifs, 371
- Methyltransferase, 113, 258, 272, 352, 371, 406, 407, 410, 418
- Microarray, 239–242, 245
  - chip, 243
  - data analysis, 244
  - databases, 243
  - experimental data, 244
  - technology, 240, 242
- Microbes, 4–6, 9, 10, 12–15, 17, 28, 29, 42, 43, 50, 58, 59, 69, 86, 87
  - assisted bioremediation, 4, 28, 29
  - activity, 5, 46
  - bioremediation process, 29
  - community structure, 25
  - degradation, 12, 28
  - diversity, 72, 86
  - inoculation, 51
  - population, 11, 16
  - strains, 30
  - technique, 239
- Microbiological contrivances, 51
- Micrococcal nuclease (MNase), 377
- Micronutrients, 79, 99, 100, 111, 441
- Microorganism development, 21
- MicroRNA (miRNA), 249, 253–262, 266–285, 321, 322, 353, 360–362, 364–367, 374, 375, 379, 384
  - microarray analysis, 365
- Mildew Locus O, 337
- Mineral
  - solubilization, 75
  - uptake, 85, 144
- Minimal/no-secondary pollution generation
  - issues, 4
- Minor allele frequency, 312
- Mitochondria, 144, 151, 153, 403–405, 407, 412, 414, 415, 421, 422
- Mitochondrial
  - activities, 414
  - assembly, 414
  - composition, 416
  - DNA, 369
  - metal concentration, 415
  - proteins, 414, 415
  - proteome, 414, 415
  - respiratory chain pathways, 415
- Mitogen-activated protein kinase, 58, 64, 141
- Mixed linear model, 311, 323
- Modern
  - agricultural system, 68
  - agriculture systems, 110

- biotechnological techniques, 74
  - tetraploid/hexaploidy wheat varieties, 190
  - Molecular
    - adaptations, 356
    - adaptive responses, 201
    - biology, 133, 187, 249, 279, 349
    - chaperones, 168, 193, 418, 420, 422
    - marker technology, 330
    - mechanism, 111, 112, 137, 157, 189, 192, 251, 252, 265, 280, 321, 382, 413, 418
    - modification, 267, 279
    - pathways, 43, 413, 436
    - plant
      - breeding, 168
      - conditions, 41
      - techniques, 181, 330, 401
  - Molybdenum, 440
  - Monodehydroascorbate reductase, 244
  - Monoubiquitination, 372
  - Morphological
    - consequences, 364
    - development, 255
    - mechanisms, 111, 113
  - mRNA degradation, 240, 278, 375
  - MspI restriction digestion, 379
  - Mucor* sp., 21, 22
  - Mucoromyceta, 79
  - Multicellular
    - animals, 24
    - plants, 372
  - Multidimensional methods, 383
  - Multifunctional genes, 194
  - Multigene transfer, 192
  - Multigenetic nature, 203
  - Multigenic nature, 190, 192, 203
  - Multiple stress
    - conditions, 87
    - resistant crop plants, 209
  - Multiprotein bridging factor 1C, 360
  - Mungbean, 102, 106, 138, 172, 173
    - resistance, 172
  - Mungbean yellow mosaic disease, 173
  - Mustard, 109, 110, 366
  - Mutualistic
    - interaction, 64
    - relationship, 20
  - Mycelial
    - network, 83
    - structure, 79
  - Mycobacterium*, 13, 14
  - Mycorrhiza, 42–44, 49, 80, 87
    - induced resistance, 87
  - Mycorrhizal
    - association, 79, 112
    - colonization, 60, 86
    - fungi, 41, 42, 60, 80, 86
  - Myoinositol, 436
- N**
- Naphthalene, 14, 22
  - Natural
    - antisense siRNA, 254, 258, 260
    - antisense transcript derived siRNAs (nat-siRNA), 249, 258, 277, 278
    - environment, 209, 239
    - methylation, 363
    - remediation techniques, 18
    - variations, 310, 319
  - Necrosis, 357, 364
  - Nematode, 25–27, 86, 175
    - community
      - indices, 26
      - structure, 25
    - tolerance mechanism, 26
  - Nematodes, 3, 5, 6, 21–28, 30, 62, 69, 86, 334
  - Next generation sequencing (NGS), 240, 305–307, 313, 315, 320, 323, 377–379
    - technology, 266, 313, 320, 321
  - Nickel, 78, 440
  - Nicotiana*, 71, 172, 272, 277, 333, 337, 338, 404, 405, 437, 440
    - benthamiana*, 172, 333, 337, 338, 404, 405
    - tabacum*, 333, 437, 440
  - Nicotianamine, 112
  - Nicotine adenine dinucleotide, 374
  - Nitrate reductase, 104, 141, 147
  - Nitric oxide, 5, 63, 104–107, 112, 115, 148, 149
  - Nitrogen, 9, 43, 46, 47, 49, 51, 59, 60, 62, 65, 72, 73, 79, 80, 83, 107, 116, 142, 146, 433, 440
    - oxygen species, 51
  - Noccaea caerulea*, 363
  - Nodule formation, 72, 79
  - Nonanucleotide sequences, 337
  - Non-catalytic functions, 28
  - Non-coding RNAs (ncRNAs), 374, 375

- Non-conserved miRNAs, 364
  - Non-enzymatic system, 155
  - Non-halophytic plants, 249
  - Non-homologous end joining, 329, 331
  - Non-indigenous, 7
  - Non-invasive
    - dry seed priming, 135
    - method, 134
  - Non-lethal physical stimulant, 134
  - Non-model plant species, 402
  - Non-phosphorylating respiratory pathways, 414
  - Non-photochemical
    - quenching, 105, 145, 149
    - reactions, 147
  - Non-primed seeds, 109
  - Nonredox metals, 111
  - Non-saline condition, 105
  - Nonsystemic irrigation, 109
  - Non-targeted metabolomics, 436
  - Nontoxic substances, 13
  - Non-toxic substrate products, 15
  - Non-transgenic
    - grapevine, 177
    - tobacco plants, 179
    - tomato, 177, 178
  - Non-uniform magnetic field, 151
  - Non-vascular plant, 65
  - Normal biological function, 263
  - Novel
    - chromatin structures, 381
    - high throughput techniques, 190
    - transgenic techniques, 174
  - Nuclear
    - magnetic resonance, 431, 434, 440, 441
    - phosphoproteins, 413
    - proteome, 413, 414
  - Nucleic acid, 99, 103, 108, 149
  - Nucleocytoplasmic transport, 413
  - Nucleoside diphosphate kinase, 415, 418
  - Nucleosomal substitution, 358
  - Nucleosome, 370
  - Nucleus, 405, 406
  - Nutrients, 5, 9, 44, 46, 48–64, 66, 67, 72, 74, 76, 79–84, 86, 100, 109, 111, 241, 367, 441
    - absorption, 136, 152
    - availability, 76, 79, 80, 82
    - cycle, 44
    - cycling, 25, 79
    - deficiency, 103
    - deficit, 61
    - mobilization, 43, 49, 51
    - resources, 42
    - uptake machinery, 66
  - Nutrition mobilization, 50
  - Nutritional deprivation, 366
- O**
- Ocimum basilicum*, 334, 338
  - Oilseed rape, 204
  - Oligonucleotides, 242
    - sequence, 243
  - Omics techniques, 383, 432, 434, 442
  - Open chromatin conformation, 353
  - Optimal priming environment, 115
  - Optimum crop production, 167
  - Oraesia excavate*, 171
  - Organelle
    - locatable reactive molecules, 404
    - proteomes, 401
  - Organic
    - acid, 21, 23, 24, 75, 112, 435, 439
    - compounds, 11
    - contaminants, 21, 24, 28
    - matter decomposition, 25
    - pollutant, 4, 6, 9, 15, 21, 22, 24, 29, 30
    - solutes, 72, 77, 81, 82
    - sources, 100
    - xenobiotics, 26
  - Organismal stress responses, 381
  - Organo-mercuriallyase, 21
  - Organomercurials, 21
  - Organophosphorus hydrolase gene, 24
  - Ornithine, 438
  - Oryza sativa*, 19, 70, 102, 142, 180, 264, 272, 317, 333–338, 366, 373, 382, 383, 406, 408, 410
  - Oscillator models, 152
  - Oscimum basilicum*, 73
  - Osmolytes, 44, 48, 83, 192, 193, 253
  - Osmoprotectants, 147, 170, 192, 193, 423
  - Osmoprotection, 308
  - Osmoregulators, 82
  - Osmotic
    - adaptation, 438
    - adjustment, 63, 81, 84, 108, 111

balance, 110  
 genes, 179  
 imbalance, 252  
 inhibition, 75  
 potential, 82, 103, 192, 420, 436  
 stress, 48, 60, 62, 66, 169, 199, 205, 252,  
   308, 309, 356, 368, 419, 423  
 genes, 179  
 Oxalic, 21  
 Oxidation, 15, 16, 23, 102, 155, 415  
 Oxidative  
   disintegration, 178  
   modification, 414  
   stress, 18–20, 68, 100, 103, 108, 110, 112,  
     136, 193, 252, 278, 356, 414–417, 422  
   associated gene, 367  
   window, 155  
 Oxidoreduction equilibrium, 367  
 Oxygen, 4, 9, 10, 15, 115, 136, 178, 244,  
   309, 366, 416–418, 422, 433, 440  
 Oxygenase, 15, 418  
 Oxygen-evolving enhancer protein, 417, 418  
   OEE1, 418  
   OEE2, 417  
 Ozone-depleting substances, 106

## P

*Paenibacillus*, 19, 73, 76  
   *polymyxa*, 73  
 Palmitic acid, 436  
*Panagrellus redivivus*, 26  
 Papaya ringspot mosaic virus type-W, 172,  
   334  
 Paramagnetic resonance model, 152  
 Paramural bodies, 144  
 Parental epigenetic imprinting, 362  
 Pathogen  
   infection, 369  
   populations, 43  
   recombination, 181  
 Pathogenesis, 50, 176, 179, 181  
 Pathogenic  
   attack, 63, 72  
   infections, 59  
 Pathogenic proteins, 179  
 Pathway  
   association tool, 319  
   targeted genomic regions, 376  
 Pectinases, 65

*Penicillium* sp., 67  
   *citrinum*, 69  
 Peroxidase, 15, 20, 68, 101, 103, 104, 114,  
   136, 141, 151, 153, 178  
 Peroxides, 136  
 Peroxisomes, 153, 403  
 Persistent organic  
   fertilizers, 4  
   pollutants, 6  
*Pestalotiopsis palmarum* BM-04, 24  
 Pesticides, 4, 6, 8, 10, 11, 13, 15, 41, 111,  
   136, 167, 283, 307  
 Petroleum hydrocarbons, 12, 15  
*Petunia*, 265  
*Phanaerochaete chrysosporium*, 14  
 Pharyngeal pumping, 26  
*Phaseolus vulgaris*, 65, 67, 142, 148, 273,  
   364  
 Phenanthrene, 14, 23, 24  
 Phenolics, 106, 112, 433, 441  
   compounds, 155, 438  
 Phenols, 63  
 Phenotypes, 267, 279, 305, 314–316, 319, 402  
 Phenotypic  
   changes, 432  
   contribution, 310  
   data, 315  
   effects, 206, 207  
   plasticity, 111, 350  
   variety, 313  
 Phenotyping, 312, 313  
 Phenylalanine, 438  
 Phenylpropanoid, 418, 440  
   biosynthesis, 418, 440  
   pathway, 418  
*Phoma*  
   *glomerata*, 67  
   *herbarum*, 66  
*Phomopsis liquidambaris*, 24  
 Phosphatases, 201, 371  
 Phosphate, 18, 19, 43, 49, 112, 201, 253,  
   335, 353, 365, 375, 409, 417, 418  
 Phosphodiesterases, 151  
 Phospholipid bilayer, 419  
 Phosphoribulokinase, 417, 418  
 Phosphorus, 9, 47, 60, 62, 71, 75, 79, 80,  
   84, 318, 363, 440  
 Phosphorylation, 201, 352, 357, 360, 371

- dephosphorylation cascade, 201
- Photochemical, 105, 145, 147, 149
- Photodormancy, 101
- Photorespiratory pathway, 414
- Photosynthesis, 62, 66, 70, 81–83, 86, 102, 104, 106, 107, 109, 111, 113, 114, 136, 142, 145, 146, 148, 149, 156, 178, 179, 309, 317–319, 357, 416, 417, 435, 439, 441
  - rate, 136, 145, 146, 156, 178, 179
  - related chloroplast protein activities, 416
- Photosynthetic
  - activity, 77, 144, 416
  - apparatus, 59, 83, 147, 416
  - capacity, 188
  - efficiency, 61, 75, 82, 84, 85, 101, 104, 105, 309
  - electron transport chain, 416
  - machineries, 437
  - machinery, 62, 416, 417
  - performance, 106, 116, 204
  - pigments, 70, 77, 85, 105, 136, 148
  - processes, 267
  - products, 49
  - rate, 81, 106, 146, 148, 319
  - traits, 318
- Photosynthetically active pigments, 59
- Photosystem, 49, 82, 85, 136, 409, 416, 417, 437
  - photosystem II (PSII), 136
- Phototropin, 419
- Phototropism, 137
- Physicochemical variables, 368
- Physiobiological properties, 51
- Physiochemical, 252
  - condition, 9
  - functions, 111
  - processes, 107
  - remediation approaches, 30
- Physiological, 41, 42, 57, 62, 65, 66, 72, 82, 86, 100, 101, 107, 108, 110, 113–115, 134, 137, 139, 147, 152, 157, 178, 187, 189, 191, 201, 240, 244, 251, 252, 255, 264, 277, 279, 283, 310, 351, 353, 356, 357, 362, 365, 384, 402, 413, 432, 435, 439, 441
  - adaptations, 110
  - drought, 435
  - functions, 66
  - genetic research, 402
- Phytic acids, 433
- Phytoalexins, 63, 72
- Phyto-augmentation, 10
- Phytochelatin, 112
- Phytoextraction, 15, 17
- Phyto-ferritin, 144
- Phytoferritin, 153
- Phytohormonal
  - imbalance, 65
  - regulation, 57, 58
- Phytohormones, 44, 58–60, 63, 66, 72, 82, 202
  - biosynthesis, 417
  - metabolisms, 147
- Phytophthora*
  - citrophthora*, 176
  - infestans*, 321
- Phytoremediation, 11, 17, 18, 20–24, 106
- Phytotoxic
  - cation species, 363
  - effects, 21
- Phytotoxicity, 20, 21, 24, 111
- Phytovolatilization, 17
- Pinus silvestris*, 364
- Piriformospora indica*, 68, 70, 77
- Pisum sativum*, 22, 78, 138, 407, 408, 414
- Piwi-interacting RNAs (piRNAs), 259
- Plant
  - acclimation, 201
  - adaptability, 352, 375
  - antioxidant enzymes, 20
  - architecture, 168, 252
  - biodiversity, 57
  - biomass, 83, 85, 148, 156, 435
  - biotechnological research, 191
  - breeders, 188, 252, 283
  - breeding, 168, 173, 189, 190, 202, 442
    - approaches, 168, 172
  - defense
    - mechanisms, 20
    - defense responses, 253
  - development, 17, 24, 30, 43, 48, 49, 285, 316, 367
  - engineering, 187, 192, 207, 210
    - experiment, 207
    - strategy, 192
  - genetic manipulations, 332

- genomes, 306
- growth
  - characteristics, 51
  - chemicals, 49
  - condition, 435
  - parameters, 156
  - promoting bacteria, 42, 46, 48
  - regulators, 43, 49
  - stimulating microorganisms (PGPM), 41–44, 51
  - promoting rhizobacteria (PGPR), 17–19, 42, 44, 46, 48, 49, 57–60, 72–77
- homeodomain, 372
- hormones, 41, 43, 99
- improvement, 333
- metabolomics research, 432
- microbes' association, 59
- microRNA database (PMRD), 280
- mitochondrial
  - functions, 415
  - proteome, 414
- modification, 245
- pathogenic nematode, 26
- physiology, 48, 81, 418, 435
- productivity, 187, 188
- responses, 144, 158, 189, 194, 252, 285, 306, 360, 402
- stress
  - adaptation, 403
  - database, 384
  - protein database, 384
  - responsiveness, 424
- survivability, 44
- symbiosis, 66
- Plasma membrane, 108, 147, 151, 309, 403, 404, 412, 419–424
- proteins, 419
- proton, 423
- purity, 419
- Plasmids, 12, 332
- Plasmodesmata, 265, 279, 403
- Plastid proteomes, 417
- Pleiotropic effects, 206
- Pleurotus*, 14
- Plutella xylostella*, 175
- Poa ampla*, 65
- Pod-filling stage, 105
- Point-source pollution, 4
- Pollution
  - abatement, 13
  - avoidance, 28
  - remediation, 30
- Polyamines, 178, 253, 308, 437, 438
  - accumulation, 309
- Polyaromatic
  - compounds, 14
  - hydrocarbons (PAHs), 5
- Polychlorinated
  - biphenyls, 5, 6, 8, 12, 14, 15
  - dibenzofurans, 6
  - dibenzo-p-dioxins, 6
- Polycarbonyl repressive complex2 (PRC2), 372
- Polycyclic aromatic hydrocarbon (PAH), 10
- Polyethylene glycol, 419
- Polyketides, 76
- Polymer matrix, 13
- Polymerase chain reaction, 241, 244, 316, 363, 377, 378
  - PCR-based approaches, 241
- Polymer-like lignin, 418
- Polypeptides, 253
- Polyphenol oxidase, 136
- Polyphenolic compounds, 309
- Ponderomotive force, 151
- Population-scale genome design, 310
- Populus*
  - euphratica*, 264, 267, 273
  - tomentosa*, 439
- Post-harvest quality of seeds, 138
- Post-priming periods, 134
- Post-transcriptional, 253, 255, 262, 266, 268, 277, 278, 280, 282, 283, 352, 367, 371, 374, 375, 379
  - activity, 255
  - gene silencing, 268, 375
  - levels, 253, 262, 278
  - modification, 253, 352, 376, 386, 420
  - regulation, 266, 268, 282
- Potassium, 67, 80, 83, 109, 244, 309, 440
- Potato, 145, 175, 176, 178, 197, 199, 204
  - plantlets, 145
- Potential
  - biocontrol agent, 76
  - commercial technology, 11
  - molecular markers, 307
- Potentially

- harmful toxic elements, 20
  - toxic compounds, 4
  - Pratylenchus penetrans*, 86
  - Precipitation, 20, 21, 179, 357, 368
  - Precision agriculture, 133
  - Pre-germinative metabolic process, 100, 108
  - Pre-sowing
    - magnetic field exposures, 144
    - magnetization, 156
  - Prevotella, 332, 337
  - Primary
    - metabolites, 433, 435, 443
    - miRNA, 260
    - stressors, 66
  - Primitive symbiotic relationship, 60
  - Production of,
    - biofertilizers, 43
    - crops, 42, 250, 357
    - metabolites, 101, 110
    - osmolytes, 59
  - Programmed cell death, 415, 422
  - Proliferation, 12, 64, 136, 256, 350
  - Prolificacy, 42
  - Proline, 18, 45–47, 63, 68, 70, 77, 82–84, 103, 105, 107, 112, 140, 147, 168, 180, 193, 278, 317, 423, 436–441
  - Prolonged heavy metal exposure, 62
  - Protease, 105, 115, 142, 147, 148, 151, 152
  - Protective
    - metabolites, 433, 436, 438
    - secondary metabolites, 76
  - Protein, 279, 402, 406–411
    - biosynthesis, 414
    - bound DNA sequences, 377
    - coding genes, 260
    - degradation, 413, 420
    - denaturation, 16, 178, 179
    - disintegration, 438
    - homeostasis, 178, 179, 267
    - interaction networks, 244, 245
    - protein interaction, 360, 403
    - repression, 255
    - RNA complexes, 264
    - synthesis, 101, 108, 113, 114, 420
    - synthesizing machinery, 114, 253
  - Proteinases, 65
  - Proteolysis, 420
  - Proteolytic proteins, 420
  - Proteome, 110, 384, 403, 404, 413–419, 422, 424
  - Proteomic
    - analysis, 402
    - strategies, 264
  - Proteomics, 240, 384, 401–404, 406, 419, 425, 434, 435
  - Protospacer adjacent Motif, 332
  - Prunus cerasifera*, 80
  - Pseudomonadaceae*, 20
  - Pseudomonas*, 13, 19, 22–24, 42, 45–48, 60, 65, 68–74, 76–78, 171, 338
    - aeruginosa*, 19, 76–78
    - fluorescens*, 65, 68, 74, 77
    - monteilii*, 73
    - polymyxa*, 76
    - putida*, 22, 24, 45, 72
    - stutzeri*, 46
    - syringae*, 171, 338
  - Pteridophytes, 257
  - Pteris vittata* L., 21
  - Public internet-based information hubs, 384
  - Pulsed
    - electromagnetic field (PMF), 139
    - magnetic field, 104
  - Putrescine, 436, 437
  - Pyrene, 14, 28
  - Pyrroline-5-carboxylate dehydrogenase, 278
- ## Q
- Quantitative
    - approach, 434
    - characters, 190
    - gene expression, 20
  - Quantitative trait loci (QTL), 170, 173, 181, 191, 314–317
    - mapping, 173, 181, 314–316
  - Quantum state, 152
  - Quinoa cultivars, 111
- ## R
- Radical pair mechanism, 152, 153
  - Radioactive substances, 14
  - Raffinose, 170, 436, 440
  - Ran-binding protein (RanBP), 406, 413
  - Random amplified polymorphic, 174, 181
  - Rate of germination, 102, 115, 142

- Reactive oxygen species, 26, 28, 45, 50, 51, 59, 62, 63, 68, 73–75, 82, 83, 102–105, 107, 108, 110, 111, 115, 136, 141, 142, 149, 153, 155, 170, 178, 201, 244, 278, 318, 364, 367, 406, 407, 413–416, 418, 420, 422, 433, 435, 437  
scavenging, 136, 155, 367, 407, 413, 414, 418  
ability, 178
- Recalcitrant compounds, 13
- Receptor kinase protein, 364
- Recombinant  
DNA technology, 168  
inbred line, 315
- Recombination frequency, 174
- Redox homeostasis, 102
- Regeneration, 104, 148, 333, 369, 416
- Regulator proteins, 423
- Regulatory  
genes, 177, 189, 192, 201, 239, 246, 320  
transcription machinery proteins, 379
- Relative electrolyte conductivity, 107
- Remediation, 4, 5, 19  
efficacy, 29  
wastewater, 15
- Repressor  
binding sites, 378  
silencing 1 (ROS1), 370
- Reproductive  
mechanism, 382  
organs, 264
- Reserve food transportation, 144
- Resistance gene identification, 242
- Respiratory electron transfer, 439
- Responsive element-binding protein, 374
- Restriction fragment length polymorphism, 174, 181, 330
- Retroelements, 262, 266
- Retrotransposons, 259, 407
- Rhizobacteria, 17, 30, 48, 49, 57, 74, 76, 79, 86, 87
- Rhizobitoxine, 43, 48, 72
- Rhizobitoxine production, 43
- Rhizobium*, 47, 48, 67, 78, 79  
*etli*, 67
- Rhizoctonia* sp., 65  
*solani*, 72
- Rhizofiltration, 17
- Rhizoglomus*  
*intraradices*, 80  
*irragularis*, 83
- Rhizosphere, 72  
bio-augmentation, 10  
microorganisms, 87
- Rhizospheric  
area, 59, 65  
bacteria, 20, 48, 60  
populations, 20  
bacterial strains, 20  
microbes, 18  
microorganism, 11
- Rhodococcus*, 13, 78
- Riboregulators, 254, 262, 264
- Ribosome integrity, 114
- Rice  
genome, 257, 319  
tungro disease resistance, 334
- RNA  
directed DNA methylation (RdDM), 258, 262, 356, 359, 360, 370, 375, 376  
induced  
silencing complex (RISC), 253, 254  
transcriptional silencing, 278  
interference, 176, 177, 250, 251, 259, 26, 266, 278, 2825, 283, 285, 351, 375  
technology, 250  
mediated modifications, 350  
polymerase, 255, 256, 260, 262, 375, 381  
II, 256, 260, 381  
IV, 375  
silencing pathways, 266
- Root  
colonization, 81  
growth, 62, 66, 73, 74, 78, 102, 146  
response, 135  
knot nematode, 175  
length, 47, 79, 101, 139, 317, 318  
metabolome profile, 438  
respiration, 366  
shoot  
development, 365  
length, 114  
water conductivity, 82



## S

- Saccharomyces cerevisiae*, 178, 372  
*Saccharum* sp., 65, 264, 274  
 S-adenosyl L methionine, 369  
 S-adenosyl-L-methionine decarboxylase, 178  
 S-adenosylmethionine synthetase 2, 408, 415  
 Sakuranin, 438  
 Salicylic acid, 45, 63, 112  
 Saline  
   environment, 49, 63, 73, 75, 356  
   soil, 48, 179, 309  
   stress, 136, 139, 179, 356  
   water irrigation, 179  
 Salinity, 24, 42, 46–49, 51, 58, 60, 61, 66–69, 72, 73, 75, 80, 81, 100, 103–105, 107, 109–111, 136, 142, 146, 156, 167, 169, 180, 196, 205, 240, 241, 245, 249–253, 266, 267, 277, 278, 280, 283–285, 307, 317, 334, 350, 355–357, 415, 416, 433, 438, 442  
   resistance of plant, 48  
   stress, 148, 308, 356  
     resistant microorganisms, 46  
     tolerance, 49  
   toleration, 46  
 Salt, 60, 62, 109, 169, 179, 180, 196–200, 205, 249, 269, 308, 317, 356, 408, 437  
   accumulation, 179  
   induced  
     stress, 249, 251, 252, 267, 277, 278, 282, 285  
     stressful conditions, 267  
   remediation, 252  
   resistance, 277, 285  
   resistant, 46, 250, 251  
     bacteria, 46  
   sensitive plants, 252, 356  
   stress, 269, 308  
     inducible gene, 278  
     responsive novels, 277  
   stressed-tolerant rice cultivars, 438  
   tolerance, 60, 68, 75, 77, 82, 104, 110, 169, 196, 197, 249, 250–252, 268, 277, 282, 317, 319, 322, 334, 415  
     genes, 268  
     responses, 251  
   tolerant  
     cultivars, 250, 252  
     plants, 252  
     tolerating cultivars, 250  
 Saplings, 365, 367  
 Savior metabolites, 100  
 Scavenger proteins, 422  
 Scavenging enzymes, 59, 108, 155, 178  
*Scutellospora heterogama*, 80  
 Secondary  
   metabolic pathways, 442  
   metabolites, 58, 66, 72, 74, 76, 112, 148, 151, 265, 309, 431, 433, 436, 438, 443  
   pollution, 30  
   stress condition, 66  
   stresses, 368  
   toxic compounds, 5  
 Second-generation sequencing, 314  
 Sedoheptulose-1,7-bisphosphatase, 409, 417  
 Seed  
   characteristics, 101  
   germination, 66, 100, 103, 105–107, 109, 110, 112, 114–116, 133–137, 142, 148, 153, 254, 257, 266, 267, 283, 309, 317, 437  
   hydration, 100, 108, 113  
   invigoration, 111, 156  
   magnetization, 135, 157  
   pre-magnetization, 136  
   pre-magnetization process, 140  
   pretreatment, 135, 139  
   priming, 99–101, 110–114, 133, 134, 146  
   protein, 106  
   vigor, 101, 109, 138, 142  
 Seedling  
   growth, 100, 103–105, 108, 110, 111, 115, 133, 139, 140, 142, 144, 148  
   vigor, 102, 106, 134–136, 142, 146  
 Senescence, 113, 356, 441  
 Sequence  
   complementarity, 256–258, 260  
   determinants, 268  
   homology, 424  
   specific nucleases (SSNs), 330  
 Sequencing-based approaches, 241  
 Serine hydroxymethyltransferase, 416  
 Serotonin, 438  
*Serratia*, 19, 21, 42, 47, 60, 68, 72  
   *plymuthica*, 72  
 Sessile  
   organisms, 250  
   plants, 349

- Setaria italica*, 140, 274  
 Shikimate, 438, 439  
 Shoot elongation rates, 112  
 Short  
   interfering RNAs, 249, 253–255, 258–260, 262, 266, 267, 277–279, 282–285, 353, 370, 374, 375, 379  
   range silencing signal, 266  
   term acute ozone treatment, 417  
 Siderophore, 19, 43, 46, 60  
 Siderophores, 21–24, 41, 49, 72  
 Signal  
   perception, 201  
   transduction, 58, 189, 191, 194, 201, 252, 351, 401, 417, 418  
   transmission, 413  
 Signaling networks, 209, 210  
 Silencing parasitic gene elements, 251  
 Silent information regulator 2, 374  
 Simple sequence repeats, 174, 181, 330  
 Single  
   gene traits, 203  
   guide RNA, 330, 332  
   nucleotide polymorphisms (SNPs), 307  
   stranded RNA, 256, 262, 353  
   structural gene engineering, 203  
 Small  
   RNA, 249–251, 253–255, 259, 260, 262–280, 282–285, 352, 368, 374, 375, 379, 380, 385, 386  
   based strategies, 264, 283  
   mediated gene silencing, 264, 284  
   sequencing, 379  
   temporal RNA (st-RNA), 259  
 Sodium, 46, 49, 67, 104, 105, 109, 110, 169, 179, 180, 356, 363  
 ion  
   extrusion, 169  
   hydrogen ion antiporters, 169  
 nitroprusside, 105, 241, 305, 307, 312, 315–319, 321, 322, 330  
 tungstate, 104  
 Soil  
   amendments, 167  
   fertility, 41, 42, 60, 79  
   microbial  
     activity, 25  
     diversity, 3, 42  
   plant-microbe interaction, 51  
   rhizosphere, 60, 76  
   salinity, 46, 250, 285  
   transmitted illnesses, 50  
*Solanum*  
   *habrochaites*, 360  
   *lycopersicum*, 77, 84, 85, 102, 139, 142–144, 336, 338, 417  
   *melongena* L., 144  
   *nigrum*, 22, 66  
   *villosum*, 110  
 SOLiD sequencing technology, 320  
 Sonication, 376  
 Soybean  
   ameliorated arsenic toxicity, 106  
   calli, 104  
   seeds, 107, 139, 145  
 Spatio-temporal regulation, 284  
 Spermine, 112, 437  
*Sphingomonas*, 13  
*Spodoptera litura*, 175  
 Static magnetic field, 102, 104–107, 134, 136, 139, 141, 142, 145–149, 153, 156, 158  
 Stochastic resonance, 152  
 Stomatal  
   closure, 205, 357, 423  
   conductance, 45, 82, 109, 145, 147–149, 319, 437  
   functions, 110  
   opening, 82  
 Storage conditions, 101  
 Strawberry mild yellow edge potyvirus, 177  
*Streptococcus pyogenes*, 332  
 Stress  
   acclimation, 380, 403  
   associated  
     genes, 352  
     proteins, 193  
   causing factors, 42  
   hormones, 81  
   induced  
     alterations, 416  
     genes, 350, 352  
     proteins, 385  
   inducible promoter, 206, 207

- memory, 100, 106, 134, 146, 358, 372, 381, 382
  - related
    - expressions, 57
    - genes, 58, 190, 194, 205, 206, 244, 306, 313, 384
    - phytohormones, 63
    - protein, 205, 403
    - siRNAs, 278
  - resistance, 101, 180, 244, 264, 278, 316, 320, 322, 376, 402
  - resistant
    - crops, 279, 385
    - cultivars, 283, 285
  - responsive
    - downstream, 195, 201, 202
    - epigenetic modification, 352
    - gene, 59, 63, 66, 68, 81, 195, 201, 253, 277, 278, 357, 358, 363, 367, 381
    - miRNAs, 277, 361, 366
    - nuclear protein expression profiles, 413
    - phytohormones, 75
    - protein production, 368
    - proteins, 108, 412, 420
    - sRNAs, 277
  - sensing, 417, 418
  - signaling, 100, 351, 418
  - specific marker, 431
  - tolerance, 60, 61, 63, 65, 67–69, 73, 74, 76, 86, 87, 100, 101, 110, 116, 139, 168, 171, 177, 180, 181, 187–192, 194, 195, 200–204, 206–209, 239, 243–245, 251, 252, 282, 285, 310, 317, 320, 336, 385, 401, 402, 413, 434, 436, 437, 440–442
    - crops, 209
    - plants, 193, 329, 330
    - related proteins, 402
    - upregulated genes, 358
  - Striga*, 86
  - Strigolactone, 49, 82
  - Stromal proteome, 417
  - Structural
    - conformations, 251
    - stability, 170
  - Subcellular
    - fractionation, 417
    - proteomic analysis, 404
    - separation, 403
  - Submergence responsive
    - miRNAs, 367
    - genes, 366
  - Subsurface bioremediation, 11
  - Sucrose, 380, 416, 417, 423, 438–440
    - non-fermenting (SNF), 380
  - Sugar
    - syntheses, 423
    - transporters, 423
  - Sulfhydryl group, 111, 112
  - Sulfidogens, 15
  - Sulfur, 8, 13, 317, 318, 440
    - deficiency, 317, 318
  - Sumoylation, 253, 352, 371
  - SUMOylation, 360
  - Superoxide, 58, 68, 70, 101, 114, 116, 136, 141, 147, 153, 155, 178, 244, 246, 270, 364, 366, 416, 418, 422
    - dismutase, 20, 58, 63, 68, 70, 76, 101, 103–105, 136, 141, 143, 149, 153, 155, 178, 244, 245, 272, 406–408, 410, 411, 414–416, 418, 422
    - ions, 136
    - molecules, 153
  - Supplemental UV-B radiation, 107
  - Suppressed cellular metabolism, 62
  - Surface mineralization, 111
  - Sustainable
    - agriculture, 58, 62, 75
    - ecosystem, 29
  - Symbiotic
    - microorganisms, 59, 60, 87
    - noninfectious microorganisms, 86
    - relationship, 59, 60, 64, 76
  - Symplast, 403, 418
    - environments, 403
  - Systematic
    - acquired resistance, 50, 86, 176, 380
    - high-throughput methodologies, 402
    - resistance, 60, 76
    - sensory, 252
- ## T
- Tailored genetic switches, 201
  - Target genomic sequence, 200
  - Technological
    - advancement, 135, 251
    - complexity, 5

- Telomeres, 283
- Temperature, 4, 5, 9, 11, 42, 60, 62, 63, 65, 66, 68, 69, 80, 81, 83, 85, 100, 101, 106–108, 112, 113, 146, 167–172, 178–180, 197, 200, 241, 307–309, 334–360, 368, 369, 433, 439, 440
  - stress, 62, 63, 69, 83, 85, 106, 112, 113, 170, 171, 178, 179, 359, 360
- Tensile-tolerant microorganisms, 43
- Terminal
  - electron acceptor, 5, 16
  - heat stress, 318
- Terrestrial
  - ecosystems, 362
  - toxicity investigations, 26
- Tetra-homomethionine, 438
- Tetranuclear cells, 144
- Tetraselmis suecica*, 334
- Theobroma cacao*, 68
- Thigmotropism, 137
- Tillering, 365
- Tissue differentiation, 73
- Tobacco
  - curly shoot virus, 172
  - mosaic virus, 337
- Tocopherols, 63
- Tolerance, 16, 19, 26, 28, 44, 48, 50, 51, 58–60, 63, 66, 68, 69, 72, 73, 80–82, 83, 99, 103, 106–110, 113, 138, 149, 169–180, 188–198, 202–206, 209, 240, 244, 245, 250–252, 268, 277, 278, 306, 317, 318, 320, 334–337, 365, 376, 402, 418, 423, 432, 433, 436, 438–440
  - mechanism, 26, 240, 245, 306, 402
  - genotypes, 167, 190
  - microorganisms, 16
- Tomato
  - leaf curl virus, 177
  - mosaic virus (TMV), 176
  - yellow leaf curl China virus resistance, 172
- Total
  - dissolved solids, 143
  - petroleum hydrocarbon, 23, 24
- Toxaphene, 6
- Toxic
  - hazardous wastes, 3
  - pollutants, 4, 9
- Traditional
  - agricultural practices, 42
  - breeding methods, 176
  - breeding strategies, 306
  - remediation approaches, 11
- Trainable genes, 381
- Trans
  - engineering, 207
    - acting siRNA, 249, 254, 258, 260, 285
    - ta-siRNA, 249, 254
  - activating crRNA, 332
- Transcellular membranes, 201
- Transcript dynamics, 277
- Transcription, 44, 47, 113, 177, 178, 201, 205, 206, 208, 210, 244, 251–253, 255, 260, 264–276, 278, 314, 350–352, 358, 359, 361, 369–373, 375–381, 384, 385, 408, 413, 415, 436
  - activator-like effector nuclease, 172
  - factor, 113, 177, 178, 205, 206, 210, 244, 251, 253, 268–276, 351, 358–361, 371, 373, 376–379, 384, 385, 413, 436
  - system plants, 253
- Transcriptional, 58, 101, 108, 189, 191, 194, 203, 207, 209, 250, 251, 253, 255, 257, 262, 266–268, 282, 351–353, 360, 361, 364, 367, 372, 374, 375, 381, 382, 386, 413
  - control, 58, 194, 413
  - expressions, 194
  - gene silencing, 268
  - machinery, 253
  - manipulations, 209
  - memory, 381, 382
  - regulators, 253, 360, 367
  - repression, 251, 268, 372
  - repressor/activator, 207
  - system, 267
- Transcriptomes, 320, 323, 383
  - analysis, 171, 240, 320
  - wide changes, 313
- Transcriptomics, 240, 264, 280, 284, 402
- Transgenerational
  - consequences, 381
  - epigenetic
    - impacts, 376
    - transmission, 350
  - gene expression memory, 382
  - memory, 110, 381, 382

Transgenes, 277  
 Transgenic apple  
   overexpressing NPR1 gene, 176  
   plants, 177  
 Transgenic  
   approaches, 168, 174–179, 189, 192  
   disease resistance, 176  
   drought stress, 177  
   heat stress, 178  
   insect pest resistance, 175  
   virus resistance, 176  
   bananas, 177  
   chili, 177, 179, 180  
   crop plants, 175  
   grapevines, 177  
   guava, 176  
   maize plants, 207  
   manipulation, 193  
   orange, 176  
   plants, 178, 179, 188, 191–193, 202, 205–207  
   overexpressing TFs, 206  
   rice  
     drought tolerant, 170  
     plant tolerant, 169  
     plants, 204  
   strawberry, 177  
   tomato, 176–178  
     plants, 171  
 Trans-Golgi network, 403  
 Transitive RNA interference, 258, 259  
 Transketolase, 418  
 Translation repression, 254  
 Translational  
   inhibition, 257  
   repression, 259, 264  
 Translocation, 21, 44, 69, 71, 84, 85, 382, 420, 441  
 Transpiration, 67, 82, 109, 113, 145, 148, 149, 319, 357, 437, 439  
 Transposable element, 284, 362, 375  
 Transposons, 266, 352, 370, 375  
 Trehalose, 63, 67, 193  
 Trehalose-6-phosphate synthase enzyme, 67  
 Triacylglycerol, 436  
 Tricarboxylic acid cycle, 415, 433, 439  
 Trichloroethylene, 14  
*Trichoderma*, 45, 68, 70, 172, 176

Trichome tissues, 112  
*Trifolium repens* L., 363  
 Trinitrotoluene, 15  
*Triticale* seeds, 105, 139  
*Triticum*, 69, 82, 84, 141, 148, 149, 180, 264, 275, 278, 317, 336, 338, 408, 409, 437, 440  
   *aestivum*, 82, 84, 141, 148, 149, 180, 317, 336, 338, 408, 409, 437, 440  
   *turgidum*, 264  
*Tritium aestivum*, 102  
 Trivial off-target activities, 333  
 Tropism, 135  
 Tru-gRNAs, 333  
 Truncated gRNAs, 333  
 Tryptophan, 153, 437  
   residue, 153  
 Tumorigenesis, 256  
 Turgor pressure water efflux, 252  
 Turnip mosaic virus, 172, 334  
 Two-phase partitioning technique, 419  
 Tyrosine, 438

## U

Ubiquitin, 353, 420  
 Ubiquitinates, 371  
 Ubiquitination, 253  
 Ubiquitinylation, 371  
 Ultra-high performance liquid chromatography, 435  
 Ultrastructural peculiarities, 144  
 Uncolonized root system, 79  
 Universal food  
   security, 51  
   stability, 41  
 Unsaturated soil, 10  
 Upstream regulatory circuits, 209  
 Urbanization, 4, 69, 306  
 UV radiation, 350, 355, 361

## V

Vacuoles, 144, 403, 423, 424  
 Valine, 308, 437, 438, 440  
 Variegated flowers, 265  
*Variovorax*, 42  
 Vascular tissue differentiation, 66  
 Vernalization, 370

*Verticillium dahliae*, 72  
 Vesicular-arbuscular mycorrhiza (VAM), 21  
*Vicia faba*, 49, 141, 143, 144, 313  
*Vigna*  
   *radiata*, 84, 102, 138, 141  
   *unguiculata*, 264, 267  
 Vigor index, 138, 144  
 Viral diseases, 176  
 Viruses, 8, 59, 62, 174, 177, 256, 277, 283, 332, 334  
*Vitis*  
   *amurensis*, 440  
   *vinifera*, 337, 437, 440  
 Volatile  
   compounds, 58  
   organic pollutants, 24  
   products, 16  
 Volatilization, 12, 49  
 Volcanic eruption, 111, 362  
 Voltage-dependent anion channel, 415

## W

Water  
   conductance, 67, 82, 84  
   deficiency stress response, 382  
   deficit  
     condition, 62  
     responsive proteins, 418  
   productivity, 144  
   related stress, 424  
   scarcity, 435  
   stress, 105  
   stressed root elongation zone, 418  
   waterlogging, 366, 368

Wet priming treatments, 101  
 Wheat, 60, 65, 70, 75, 76, 80, 102, 104, 105, 109, 110, 112, 138, 139, 146, 151, 168, 170, 172, 174, 188, 191, 204, 207, 245, 311, 312, 316, 320, 337, 356, 362, 368, 414–417, 439  
 Whole-genome  
   bisulfite sequencing (WGBS), 378  
   sequence, 283

## X

Xanthine oxidase, 153  
 Xenobiotic  
   catabolic genes, 20  
   compounds, 12  
   species, 7  
   substances, 15, 25  
 Xenobiotics, 28  
*Xiphinema vuittenezi*, 26  
 Xylose, 439

## Y

Yellowstone National Park, 69

## Z

*Zea mays*, 19, 70, 82–85, 102, 139, 142, 145, 148, 149, 264, 276, 336, 409, 410  
 Zinc, 18, 49, 144, 172, 200, 208, 270, 372, 374, 407, 413, 440  
   finger, 200, 208, 172, 372, 407  
     domains functions, 208  
     nuclease, 172  
 Zucchini yellow mosaic virus, 172, 177, 334