Chittaranjan Kole *Editor*

Genomic Designing for Abiotic Stress Resistant Cereal Crops

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Editor
Chittaranjan Kole
Raja Ramanna Fellow
Department of Atomic Energy
Government of India
ICAR-National Institute for Plant Biotechnology
New Delhi, India

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Dedicated to

Dr. Gurdev S. Khush, FRS

Member, US National Academy of Sciences; Adjunct Professor Emeritus, University of California, Davis; and Former Head, Plant Breeding, Genetics and Biotechnology, International Rice Research Institute

With regards & gratitude for his generous appreciations of my scientific contributions and service to the global academic community, and his constant support and encouragement during my professional journey!

Preface

Crop production is drastically affected due to external or environmental stresses. The biotic stresses cause significant yield losses in the range of 31–42% together with 6–20% loss during the post-harvest stage. The abiotic stresses also aggravate the situation with crop damage in the range of 6–20%. Understanding the mechanisms of interaction of plants with the biotic stresses caused by insects, bacteria, fungi, viruses, oomycetes, etc., and abiotic stresses due to heat, cold, drought, flooding, submergence, salinity, acidity, etc., is critical to develop resilient crop varieties. Global warming and climate change are also causing emergence of new diseases and insects together with newer biotypes and physiological races of the causal agents on the one hand and aggravating the abiotic stress problems with additional extremes and unpredictability. Development of crop varieties resistant and/or adaptive to these stresses is highly important. The future mission of crop improvement should, therefore, lay emphasis on the development of crop varieties with optimum genome plasticity by possessing resistance or tolerance to multiple biotic and abiotic stresses simultaneously. A moderate estimation of world population by 2050 is about 9.3 billion that would necessitate an increase of crop production by about 70%. On the other hand, the additional losses due to climate change and global warming somewhere in the range of 10–15% should be minimized. Therefore, increase in the crop yield as well as minimization of its loss should be practiced simultaneously focusing on both 'adaptation' and 'mitigation.'

Traditional plant breeding practiced in the last century contributed a lot to the science of crop genetic improvement. Classical plant breeding methods including selection, hybridization, polyploidy and mutation effectively catered to the basic F^5 needs—food, feed, fiber, fuel and furniture. The advent of molecular breeding and genetic engineering in the latter part of twentieth century complimented classical breeding that addressed the increasing needs of the world. The twenty-first century came with a gift to the geneticists and plant breeders with the strategy of genome sequencing in Arabidopsis and rice followed by the tools of genomics-aided breeding. More recently, another revolutionary technique, genome or gene editing, became available for genetic correction of crop genomes! The travel from 'plant breeding' based on visual or perceivable selection to 'molecular breeding' assisted

by linked markers to 'transgenic breeding' using genetic transformation with alien genes to 'genomics-aided breeding' facilitated by known gene sequences has now arrived at the age of 'genetic rectification' employing genome or gene editing.

Knowledge on the advanced genetic and genomic crop improvement strategies including molecular breeding, transgenics, genomic-assisted breeding and the recently emerged genome editing for developing resistant, tolerant and/or adaptive crop varieties is useful to students, faculties and scientists in the public and private universities and organizations. Whole-genome sequencing of most of the major crop plants followed by genotyping-by-sequencing has facilitated identification of exactly the genes conferring resistance, tolerance or adaptability leading to gene discovery, allele mining and shuttle breeding which in turn opened up the scope for 'designing' or 'tailoring' crop genomes with resistance/tolerance to biotic and abiotic stresses.

To my mind, the mission of agriculture in this century is FHNEE security meaning food, health, nutrition, energy and environment security. Hence, genome designing of crops should focus on breeding of varieties with higher yields and improved qualities of the five basic F5 utilities; nutritional and neutraceutical compounds; and other industrially and aesthetically important products and possibility of multiple utilities. For this purpose of 'precise' breeding, employment of the genetic and genomic techniques individually or in combination as and when required will play a crucial role.

The chapters of the 12 volumes of this twin book series entitled *Genomic Designing for Biotic Stress Resistant Crops* and *Genomic Designing for Abiotic Stress Resistant Crops* will deliberate on different types of biotic and abiotic stresses and their effects on and interaction with crop plants; will enumerate the available genetic diversity with regard to biotic or abiotic stress resistance among cultivars; will illuminate on the potential gene pools for utilization in interspecific gene transfer; will brief on the classical genetics of stress resistance and traditional breeding for transferring them to their cultivated counterparts; will discuss on molecular mapping of genes and QTLs underlying stress resistance and their marker-assisted introgression into elite crop varieties; will enunciate different emerging genomics-aided techniques including genomic selection, allele mining, gene discovery and gene pyramiding for developing smart crop varieties with genetic potential to produce F^5 of higher quantity and quality; and also will elaborate the case studies on genome editing focusing on specific genes. Most of these chapters will discuss on the success stories of genetic engineering in the relevant crops specifically for generating crops with resistance and/or adaptability to diseases, insects and abiotic stresses.

There are obviously a number of reviews and books on the individual aspects of plant molecular breeding, genetic engineering and genomics-aided breeding on crops or on agro-economic traits which includes the 100-plus books edited by me. However, there is no comprehensive reviews or books available that has coverage on crop commodity groups including cereals and millets, oilseeds, pulses, fruits and nuts, vegetables and technical or industrial crops, and modern strategies in single

volumes with precise focuses on biotic and abiotic stresses. The present volumes will fill this gap with deliberations on about 120 important crops or their groups.

This volume on "*Genomic Designing for Abiotic Stress Resistant Cereal Crops*" includes eight chapters on Rice, Wheat, Maize, Barley, Sorghum, Pearl Millet, Foxtail Millet and Finger Millet contributed by 61 scientists from six countries including Bangladesh, Egypt, India, Indonesia, Italy and Mexico. I remain immensely thankful for their highly useful contributions.

I am indebted to my wife Phullara who as always has assisted me directly in editing these books and indirectly through maintaining an academic ambience to pursue my efforts for science and society pleasantly and peacefully.

New Delhi, India Chittaranjan Kole

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Contributors

T. P. Ajeesh Krishna Division of Plant Biotechnology, Entomology Research Institute, Loyola College, University of Madras, Chennai, India

Supriya Ambawat ICAR-AICRP on Pearl Millet, Mandor, Agriculture University, Jodhpur, Rajasthan, India

S. Antony Ceasar Division of Plant Biotechnology, Entomology Research Institute, Loyola College, University of Madras, Chennai, India;
Division of Plant Molecular Biology and Biotechnology, Department of Biosciences, Rajagiri College of Social Sciences, Kalamassery, Kochi, India

K. A. Apoorva Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Raffaella Battaglia CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola D'Arda, Italy

Jayant S. Bhat Regional Research Centre (ICAR-Indian Agricultural Research Institute), Dharwad, Karnataka, India

Akshaya Biswal Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico

Luigi Cattivelli CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola D'Arda, Italy

G. K. Chikkappa ICAR-India Institute of Maize Research, PUSA Campus, New Delhi, India

Tatik Chikmawati Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB University), Bogor, Indonesia

Viswanathan Chinnusamy ICAR-Indian Agricultural Research Institute, New Delhi, India

Cristina Crosatti CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola D'Arda, Italy

A. Daspute College of Agricultural Biotechnology, Ahmednagar, Maharashtra, India

M. Doddamani Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

M. Faizan Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

B. Fakrudin Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Md Farhad TERI School of Advanced Studies, New Delhi, India;
Bangladesh Wheat and Maize Research Institute, Dinajpur, Bangladesh

Miftahul Huda Fendiyanto Department of Biology, Faculty of Military Mathematics and Natural Sciences, Indonesia Defense University (IDU), Komplek Indonesia Peace and Security Center (IPSC) Sentul, Bogor, Indonesia

Agostino Fricano CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola D'Arda, Italy

R. N. Gadag Division of Genetics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

S. P. Gautham Suresh Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Prakash Gopalareddy Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Aurag Gowda Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Davide Guerra CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola D'Arda, Italy

Raghavendra Gunnaiah Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

A. Hadimani Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

S. Ignacimuthu Xavier Research Foundation, St Xavier's College, Palayamkottai, India

S. Kadam Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

J. Khan Center for Cellular and Molecular Platforms, National Center for Biological Sciences, Bengaluru, India

Ramesh Kumar ICAR-India Institute of Maize Research, PAU Campus, Ludhiana, India

Sudhir Kumar ICAR-Indian Agricultural Research Institute, New Delhi, India

Uttam Kumar Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico;
Borlaug Institute for South Asia, Ludhiana, Punjab, India

T. N. Lakshmidevamma Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Charu Lata CSIR-National Institute of Science Communication and Information Resources, New Delhi, India

T. Maharajan Division of Plant Biotechnology, Entomology Research Institute, Loyola College, University of Madras, Chennai, India

M. N. Mamathashree Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Erica Mica CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola D'Arda, Italy

Miftahudin Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB University), Bogor, Indonesia

Suchismita Mondal Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico

Ganapati Mukri Division of Genetics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

Mehanathan Muthamilarasan Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana, India

Gopalakrishna K. Naidu All India Coordinated Research Project on Maize, University of Agricultural Sciences, Dharwad, India

J. Navaneetha Krishnan TERI School of Advanced Studies, New Delhi, India;
Punjab Agricultural University, Ludhiana, Punjab, India

M. L. Nithyashree Division of Agricultural Economics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

K. Omkar Babu Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

Lydia Pramitha School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

Sumi Rana Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana, India

K. Rashmi Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Ahmed Sallam Department of Genetics, Faculty of Agriculture, Assiut University, Asyut, Egypt

Rizky Dwi Satrio Department of Biology, Faculty of Military Mathematics and Natural Sciences, Indonesia Defense University (IDU), Komplek Indonesia Peace and Security Center (IPSC) Sentul, Bogor, Indonesia

Deepmala Sehgal Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico

Seema Sheoran ICAR-India Institute of Maize Research, PAU Campus, Ludhiana, India

Rakesh K. Srivastava International Crops Research Institute for Semi Arid Tropics, Patancheru, Telangana, India

Sivakumar Sukumaran Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico

C. Tara Satyavathi ICAR-AICRP on Pearl Millet, Mandor, Agriculture University, Jodhpur, Rajasthan, India

Shalini Tiwari CSIR-National Botanical Research Institute, Lucknow, India

Alessandro Tondelli CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola D'Arda, Italy

Turhadi Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB), Bogor, Indonesia

J. Ugalat Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru, India

Sarita Yadav ICAR-National Institute for Plant Biotechnology, PUSA Campus, New Delhi, India

Sunil Kumar Yadav Department of Biophysics, University of Delhi South Campus, New Delhi, India

Pranjal Yadava Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi, India

Abbreviations

AAPK	Abscisic acid-activated protein kinase
ABA	Abscicic acid
ABF	ABA-binding factor
ABF3	ABF transcription factor
ABRE	ABA-responsive element
ADC	Arginine decarboxylase
AFLP	Amplified fragment length polymorphism
ALMT1	Al-activated malate transporter
ALS1	Aluminum sensitive 1
ALS3	Aluminum sensitive 3
Alt1	Al tolerance gene 1
AM	Association mapping
AMF	Arbuscular mycorrhizal fungi
AP2	*APETALA2/Apetala2* (gene)
APX	Ascorbate peroxidase
AREB	ABA-responsive cis-element binding protein
ART1	Al resistance transcription factor 1
ART2	Al resistance transcription factor 2
ASI	Anther silking interval
ASR1	Abscisic acid, stress and ripening 1
ASR5	Abscisic acid, stress and ripening 5
AtEPFL9	*Arabidopsis thaliana* epidermal patterning factor like-9
AtHAT1	*Arabidopsis thaliana* histone acetyltransferase 1
BAC	Bacterial artificial chromosome
bHLH	Basic helix–loop–helix
bHLHU8	bHLH transcription factor
BV	Breeding value
BWMRI	Bangladesh Wheat and Maize Research Institute

bZIP	Basic leucine zipper
CaM	Calmodulin
CaMK	CaM-dependent protein kinase
CaO-NP	Calcium oxide nanoparticle
CAPS	Cleaved amplified polymorphic sequence
Cas	CRISPR-associated protein
Cas9	CRISPR-associated protein 9
CAT	Catalase
CAX1	Ca2+/H+ antiporter
CBF	C-repeat binding factor
CBF4	AP2/ERF transcription factor CB4
CBL	Calcineurin B-like protein
CCaMK	Ca2+/CaM-dependent protein kinase
CDPK	Ca2+ dependent and CaM-independent protein kinase
CDPK1	Calcium-dependent protein kinase 1
CDPK12	Calcium-dependent protein kinase 12
CDPK7	Calcium-dependent protein kinase 7
CEC	Cation-exchange capacity
CG	Candidate gene
CGBS	Conventional GBS
CIM	Composite interval mapping
CIMMYT	International Maize and Wheat Improvement Center
CIPK	CBL interacting protein kinase
CNP	Chitosan nanoparticle
COR	Cold regulated
CP-ANN	Counter-propagation artificial neural network
cpGRs	Chloroplast glutathione reductases
CPK4	Calcium-dependent protein kinase 4
CRISPR	Clustered regularly interspaced short palindromic repeat
CRISPRa	CRISPR-activated
CRK	CDPK-related protein kinase
crRNA	CRISPR RNA
CT	Canopy temperature
CWR	Crop wild relative
DA	Drought avoidance
DArT	Diversity arrays technology
ddRAD-seq	Double digest restriction-site-associated DNA sequencing
DE	Differentially expressed
DEG	Differentially expressed gene
DERF1	AP2/ERF transcription factor 1
DGE	Differential gene expression
DHN	Dehydrin
DMA	2'-deoxymugineic acid

Dof	DNA binding with one finger only
DREB	Dehydration-responsive element binding
DREB1	AP2/ERF transcription factor DRE1
DREB2	AP2/ERF transcription factor DRE2
DRIP1	DREB2A-interacting protein1
DSB	Double-stranded break
DT	Drought tolerance
DTMA	Drought Tolerant Maize for Africa
DUS	Distinctness, uniformity and stability
EcGBF3	G-box binding factor 3
EcHNRT2	*E. coracana* high-affinity nitrate transporter 2
EcLNRT1	*E. coracana* low-affinity nitrate transporter 1
ELIP	Early light-inducible protein
ELWR	Excised leaf water retention
EMS	Ethyl methanesulfonate
ENM	Engineered nanomaterial
EPF	Epidermal patterning factor
epiHDMA	3-Epihydroxy-2'-deoxymugineic acid
epiHMA	3-3-Epihidroximugineic acid
epiRIL	Epigenetic recombinant inbred line
ERA	Enhanced response to ABA
ERE	Ethylene-responsive element
ERF	Ethylene response factor
ERF10a	AP2/ERF transcription factor 10a
ERF4a	AP2/ERF transcription factor 4a
EST	Expressed sequence tag
EW	Epicuticular wax
F_2	Second filial generation
FAO	Food and Agriculture Organization
FBP	Fructose-1,6-bisphosphate
FCR	Ferric-chelate reductase
Fd-GOGAT	Ferredoxin-dependent-glutamine oxoglutarate aminotransferase
Fm	Maximal chlorophyll fluorescence
FPS	Farnesyl pyrophosphate synthase
FR	Frost resistance
FRDL4	Ferric reductase defective3-like 4
FRO	Ferric chelate reductase
Fv	Variable chlorophyll fluorescence
G x E	Genotype x environment
GAB	Genomics-assisted breeding
GB	Glycinebetaine
GBLUP	Genomic best linear unbiased prediction
GBS	Genotyping-by-sequencing
GCA	General combining ability
GCP	Generation Challenge Program

GE	Genome editing
GEBV	Genomic estimated breeding value
GERLP	Gene encoding ribosomal L32-like protein
GO	Gene ontology
GPX	Guaiacol peroxidase
GR	Glutathione reductase
gRNA	Guide RNA
GS	Genomic selection/glutamine synthetase
GSH	Reduced glutathione reductase
GSNOR	S-nitrosoglutathione-reductase
GWAS	Genome-wide association study/studies
H^2	Heritability
HARDY	AP2/ERF transcription factor DY
HDR	Homology directed repair
HeDWIC	Heat and Drought Wheat Improvement Consortium
HKT	High-affinity K+ transporter
HRF1	Harpin protein
HSF	Heat shock factor
HSP	Heat shock protein
HTMA	Heat Tolerant Maize for Asia
HTP	High-throughput phenotyping
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
ILP	Intron length polymorphism
ILs	Introgression lines
INRA	National Institute for Agricultural Research
IPCC	Intergovernmental Panel on Climate Change
IRAP	Inter-retrotransposon amplified polymorphism
IRRI	International Rice Research Institute
IRT1	Iron-regulated transporter 1
ISBP	Insertion site-based polymorphism
ISSR	Inter-simple sequence repeat
IWYP	International Wheat Yield Partnership
JA	Jasmonic acid
JERF1	AP2/ERF transcription factor J1
JERF3	AP2/ERF transcription factor J3
JW	Juice weight
LD	Linkage disequilibrium
LMM	Linear mixed effects model
lncRNA	Long noncoding RNA
LOD	Logarithm of the odds
LRL	Lateral root length
MABB/MABCB	Marker-assisted backcross breeding

MABC	Marker-assisted backcrossing
MAGIC	Multi-parent advanced generation intercross populations
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MDA	Malondialdehyde
MgO-NP	Magnesium oxide nanoparticle
miRNA	MicroRNA
ML	Machine learning
MQTL	Meta QTL
MT	Metallothionein
MTA	Marker–trait association
MT-GS	Multi-trait genomic selection
MYB2	MYB transcription factor 2
NA	Nicotinamide
NAC5	NAC transcription factor 5
NAC6	NAC transcription factor 6
NAC9	NAC transcription factor 9
NAC10	NAC transcription factor 10
NADH-NR	Nicotinamide adenine dinucleotide hydride-nitrate reductase
NAM	Nested association mapping
NBPGR	National Bureau of Plant Genetic Resources
NCED	9-cis-epoxycarotenoid dioxygenase
NCED2	9-cis-epoxycarotenoid dioxygenase 2
NDVI	Normalized difference vegetation index
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
NILs	Near-isogenic lines
NLR	Number of lateral root
Nramp	Natural resistance-associated macrophage protein
Nrat1	Nramp Al transporter 1
OECD	Organisation for Economic Co-operation and Development
OPVs	Open pollinated varieties
OsABH	Alpha/beta hydrolase family protein
OsALS1	*O. sativa*-ABC Al-sensitive 1
OsFer	Rice ferritin gene
OsIRO3	Iron transporter gene
OsMPPN	Mitotic phosphoprotein N' end family protein
OsSUI1	Translation initiation factor SUI1 domain containing protein
P5CS	Pyrroline-5-carboxylate synthase
P5CS1	Pyrroline-5-carboxylate synthase 1
PAM	Protospacer adjacent motif
PBF	Prolamin binding factor
PEG	Polyethylene glycol
PEP	Phosphoenolpyruvate
PEPRK	Phosphoenolpyruvate carboxylase kinase-related kinase

PH	Plant height
PHT1	Phosphate transporter 1
Pi	Inorganic phosphate
PKS	Phytochrome kinase substrate-like protein
PMiGAP	Pearl millet inbred germplasm association panel
PNHI	Panicle harvest index
POX	Peroxide dismutase
PP2A	Protein phosphatase 2A
PP2C	Protein phosphatase 2C
PPO	Protoporphyrinogen oxidase
PRL	Primary root length
PRO	Peroxidase
PS	Phytosiderophore
PSII	Photosystem II
PVE	Phenotypic variation explained
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
RAPD	Random amplification of polymorphic DNA
RBIP	Retrotransposon-based insertion polymorphism
RCAR	Regulatory component of ABA response
RFLP	Restriction fragment length polymorphism
RILs	Recombinant inbred lines
RJJM	Repeat junction–junction marker
RJM	Repeat junction marker
RLI	Root length inhibition
RMAP	Retrotransposon microsatellite amplified polymorphism
RNAi	RNA interference
RNAseq	RNA-sequencing
RNP	Ribonucleoprotein
ROS	Reactive oxygen species
RRG	Root re-growth
RRL	Relative root length
RUBISCO	Ribulose-1,5-bisphosphate carboxylase–oxygenase
RWC	Relative water content
SAP	Sorghum association panel
SCA	Specific combining ability
SCP	Sorghum conversion program
SD	Stem diameter
SFW	Stem fresh weight
sgRNA	Single-guide RNA
SI	Salt tolerance index
SIPK	SOS3 interacting protein kinase
SKN	Supervised Kohonen network
SNP	Single-nucleotide polymorphism
SnRK	Sucrose non-fermenting-1-related kinase

SnRK2	Sucrose non-fermenting-1-related protein kinase 2
SOD	Superoxide dismutase
SOS	Salt overly sensitive
SPAR	Single primer amplification reaction
SQR	Shan qui red
SRL	Seminal root length
SRO1c	Reactive oxygen species scavenging
SSA	Sub-Saharan Africa
SSD	Single seed descent
SSH	Stress-responsive suppression subtractive hybridization
SSN	Site-specific nuclease
SSR	Simple sequence repeat
STAR1	Sensitive to Al rhizotoxicity1
STAR2	Sensitive to Al rhizotoxicity2
STI	Salinity tolerance index
STS	Sequence tagged site
TaDREB2	Dehydration-responsive element binding protein 2
TaERF3	Ethylene-responsive factor 3
TAF6	TATA-box binding protein associated factor 6
TALEN	Transcription activator-like effector nuclease
TB	Total biomass
TE	Transposable element
TF	Transcription factor
TGBS	Tunable GBS
TILLING	Targeted induced local lesions in genome
TP	Training population
TPC1	Two pore channel1
TR	Transcriptional regulator
TRL	Total root length
TSRF1	Transcription factor 1a
USDA	United States Department of Agriculture
UTLIEF	Ultrathin-layer isoelectric focusing
VP	Validation population
V-PPase	Vacuolar H+-inorganic pyrophosphatase
VRN	Vernalization
WEMA	Water efficient maize for Africa
WGRS	Whole-genome resequencing
WGS	Whole-genome sequence/sequencing
WRKY22	WRKY family transcription factor 22
WRKY30	WRKY family transcription factor 30
WSC	Water-soluble carbohydrate
WSI	Water stress-induced
WUE	Water use efficiency
XY-F	XY-fused networks
ZFN	Zinc-finger nuclease

ZFP10	Zinc-finger transcription factor 10
ZFP182	Zinc-finger transcription factor 182
ZFP245	Zinc-finger transcription factor 245
ZFP252	Zinc-finger transcription factor 252
ZnO-NP	Zinc oxide nanoparticle

Chapter 1
Genomic Improvement of Rice for Drought, Aluminum, and Iron Toxicity Stress Tolerance

Miftahudin, Miftahul Huda Fendiyanto, Rizky Dwi Satrio, Turhadi, and Tatik Chikmawati

Abstract The opportunity of plants to escape from unwanted environments is almost nonexistent due to their sessile characteristic. Drought, aluminum (Al), and iron (Fe) toxicity under acid soil conditions are the major constraints as abiotic stresses in rice cultivation, particularly in tropical areas. These abiotic stress tolerance mechanisms are contributed by morphological, physiological, biochemical, and anatomical alterations that affect yield. The level of tolerance to these abiotic stresses is inherited quantitatively and controlled by several genes as quantitative trait loci. The objectives of this review were to highlight the current progress in investigating genes responsible for the drought, Al, and Fe toxicity, and their utilization for genomic improvement in rice. The mechanisms at the levels of morphology, physiology, biochemistry, anatomy, and particularly at the molecular level were discussed in the review. Overall, this review presents a systemic brief of drought, Al, and Fe tolerance mechanisms, recent progress in exploring genes responsible for these traits to the latest innovation in the genomic improvement of high-yielding multi-tolerant rice variety. This review could assist as guidelines for researchers and rice breeders.

Keywords Aluminum toxicity · Drought stress · Genomic · Iron toxicity · Rice

Miftahudin (✉) · T. Chikmawati
Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB University), Kampus IPB Dramaga, Bogor 16680, Indonesia
e-mail: miftahudin@apps.ipb.ac.id

M. H. Fendiyanto · R. D. Satrio
Department of Biology, Faculty of Military Mathematics and Natural Sciences, Indonesia Defense University (IDU), Komplek Indonesia Peace and Security Center (IPSC) Sentul, Bogor 16810, Indonesia

Turhadi
Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB), Jl. Taman Kencana No. 1, Bogor 16128, Indonesia

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1.1 Introduction

Rice is the main staple food for about half of the world's population. After the success of the Green Revolution, global per capita consumption of rice grew from 50 to 65 kg per year. Particularly in Asia, per capita rice consumption raised from 85 to almost 103 kg in the 1960s to 1990s. Rice consumption globally grew from 150 to 350 million metric tons (MMT) during this time. This pattern is expected to have a balance and limit rice demand to 501 MMT compared to 502 MMT supply in 2021–2022 (Wailes and Chavez 2012).

Increasing consumption and demand for rice must be accompanied by increasing rice production and productivity. While rice productivity is still increasing, the growth rate of rice production has decreased. The rice growth production was 2.5% year-on-year between 1962 and 1979, then decreased to 1.4% between 1980 and 2011 (Adjao and Staatz 2015). The Food and Agriculture Organization (FAO) stated that cereal production in April–June 2015 was contributed by rice, wheat, and coarse grains of 500, 723, and 1,300 MMT, respectively. Globally, between 1961 and 2007, the share of rice particularly in total cereal production did not change substantially, beginning from 24.6% and eventually exceeding 28.1% (Timmer 2010), so about a quarter of the world's cereal supply was rice.

The rice-harvested area is likely to be 160 million hectares in 2015–2016, and it will not change much by 2021–2022. About 80% of the total global rice area is located in several Asian countries, i.e., China, Indonesia, India, Bangladesh, Philippines, Vietnam, Thailand, and Myanmar. Asia as a whole has 90% of the rice region of the world. In comparison, Africa has only 5% (IRRI 2006), along with about 5% in Latin America (Pulver et al. 2010). In Africa, the growth rate in the rice area was 3.1% per year during 1980–2011, compared to only 0.4% per year in Asia (Adjao and Staatz 2015). Asia's share of world rice production in 2010–2021 may drop slightly from 89.9 to 89.3% (Wailes and Chavez 2012).

Conversion of land use for housing and industry, along with the limited availability of arable land, is an obstacle in increasing the growth of rice production in Asia, particularly in Indonesia. Rice extensification on marginal land may be a solution, but the climatic and edaphic conditions limit the growth and productivity of rice. Drought, aluminum, and iron toxicity in acid soils are the major constraints in rice cultivation on suboptimum-marginal land.

Rice is classified as one of the most drought-susceptible plants, due to its narrow root architecture, thin cuticle, and dynamic stomatal closing. Almost 23 million hectares of rain-fed rice are reportedly faced with drought stress (Serraj et al. 2011). Globally, the rise in drought severity combined with the lack of drought-resistant high-yielding varieties suitable for water-limitation conditions are the most limiting factors responsible for rice production. Consequently, rice cultivation is seasonal. The decline in water availability due to the lack of groundwater supplies is impacting rice production. Extreme environmental fluctuations caused by global climate change will affect most agricultural lands (Myers et al. 2017) including land for rice cultivation.

For plants, due to their sessile characteristic, the opportunity to escape the drought crisis is almost non-existent. At all stages, drought stress can be deleterious to plant growth and development. The effects of a water deficit during the reproductive stage causes male sterility and embryo abortion, resulting in low yield (Ozga et al. 2016). Understanding how rice responds to water-limiting conditions is essential for the genetic improvement of drought-stress tolerant and high-yielding rice cultivars.

Acid soils are predominantly distributed throughout the world and are a problem especially in agriculture. Acid soils are mainly distributed in two belts, i.e., in North America, South Asia, and Russia with temperate climate to dry types, and rainy tropical routes covering Southeast Asia, Australia, South America, and South Africa (Von Uexkuell and Mutert 1995). Acid soils covering an area of up to 3,950 million ha (Von Uexkuell and Mutert 1995; Bian et al. 2013) affect agricultural land in varying percentages, i.e., 38, 20, 31, 56, and 50% in Southeast Asia, East Asia, South America, North Africa, and North America, respectively (Wood et al. 2000; Hoekenga et al. 2003). A total of 239 million ha of acid soils are found in Australia and New Zealand (Von Uexkuell and Mutert 1995). Additionally, 212 million ha of acid soils were found in China and India (Bian et al. 2013). In South America, 1616 million ha of acid soils were also reported (Bian et al. 2013). In tropical areas, e.g., Indonesia, Mulyani et al. (2004) reported the total area of dryland is around 148 million ha that can be classified into 102.8 million ha of acid dryland and 45.2 million ha non-acid dryland.

Acid soil can be classified into two types, i.e., acid dryland and acid wetland. Acid dryland is a land that has properties such as low pH, cation exchange capacity (CEC), alkaline saturation and low organic C; high aluminum content (Al-saturation), P fixation, iron, and manganese content; sensitive to erosion, and poor biotic elements (Adiningsih and Sudjadi 1993; Soepardi 2001). Climate variations and relatively high rainfall result in an intensive level of alkaline leaching in the soil so that the alkaline content is low and the soil becomes acidic (Subagyo et al. 2000). This is why most of the soils in dryland have high acidity (pH 4.6–5.5) and poor nutrient content. Another type of acid soil is an acid wetland, which is found as paddy fields originating from advanced weathered mineral materials and on swamps that are mainly as acid sulfate soils and organic soils (peat). Swamplands in tropical areas, i.e., in Indonesia, are quite extensive, around 33.4–39.4 million ha (Widjaja-Adhi et al. 2000), spreading predominantly in Sumatra, Kalimantan, Sulawesi, and Papua. The swamps consist of 23.1 million ha of tidal swamps and 13.3 million ha of lowland swamps (Subagyo and Widjaja-Adhi 1998).

Intropical areas, particularly in Indonesia, acid soils are also a major problem in rice cultivation, whether on dry or wetland. Acidy dryland is in the order Ultisols, Inceptisols, Oxisols, Entisols, and a few Spodosols. Of the total acid dryland of 102.8 million ha, the largest is in the Ultisols and Inceptisols orders, with the dominant distribution in Sumatra, Kalimantan, and Papua. For acid wetlands, especially swamps, around 34.7 million ha were found, which came from mineral soils covering 22.8 million ha and peatlands of around 11.9 million ha. Mineral

soils are generally dominated by Inceptisols (Endoaquepts, Sulfaquepts) and Entisols (Hidraquents). While peat soils are dominated by Histosols (Haplohemists, Haplosafrists, and Sulfihemists). Swampy lands are spread across four major islands, namely Sumatra, Kalimantan, Sulawesi, and Papua with a total area of 33.4 million ha or 17% of Indonesia's total land area (188.2 million ha) (Nugroho et al. 1991).

According to Ritung et al. (2015) land is divided into three types, namely dry land, swampland, and non-swamp land. The increasing human population that needs settlements and other related infrastructures cause a reduction offertile agricultural land, therefore swampland can be an alternative solution to solve the decreasing agricultural land area. Swamplands are land types with stagnant continuously or seasonally submerged with water conditions. The problems faced in this type of land are acidic soil and high iron (Fe) content that triggers toxicity in plants, hence reduces the yield. Several areas in the world, especially in Asia and Africa, that have soil with high Fe content, are Vietnam (Mekong Delta), Thailand, Philippines, Indonesia, Sri Lanka, Liberia, Senegal, Burundi, Madagascar, Guinea, and Côte d'Ivoire (Becker and Asch 2005). The Fe content of the soil in those areas varies from 20 to 2,500 mg Fe^{2+}/L and could decrease rice yield ranges of 15–100%.

Since the 1980s, several studies on rice cultivation on tidal swamplands have been reported in Kalimantan, Indonesia (Noorsyamsi et al. 1984; Watson 1984), South Sumatera, Indonesia (Carew 1984; Koswara and Rumawas 1984), Bangladesh (Hamid and Islam 1984), Thailand (Arunin and Hillerislambers 1984), Samborondon, Ecuador (Johnson et al. 1984), Sri Lanka (Jayawardena 1984), West Bengal, India (Sinha and Bandyopadhyay 1984). Several rice varieties that have been cultivated by farmers in tidal swamp areas, South Sumatra, Indonesia, such as Nugu, Duku, Suwarambe, Kuatik, and Ampai were reported to have a productivity of 1.0–2.0 ton/ha (Koswara and Rumawas 1984). Apart from Indonesia, a variation of local (traditional) and modern rice productivity when cultivated on tidal swamp areas have been reported, such as the rice productivity of 2.1–3.6 ton/ha in Bangladesh (Hamid and Islam 1984), 1.2–3.5 ton/ha in Thailand (Arunin and Hillerislambers 1984), and 1.8–7.5 ton/ha in Samborondon, Ecuador (Johnson et al. 1984). Research conducted in the dry season of 2018 reported that several Indonesian swamp rice varieties cultivated in the tidal swamp of Barito Kuala Regency, South Kalimantan (tidal swamp type B overflow) had a productivity of 2.5–5.8 ton/ha with the highest productivity achieved by rice cv. Inpara 2 (Ningsih et al. 2020). The variation of rice productivity in tidal swampland mostly depends on the ability of rice cultivar to adapt to soil type with high Fe. Therefore, designing new rice genotypes that tolerant of the condition is an important task to provide new rice varieties that produce a high yield in such soil conditions.

Based on the study of soil categories, either acid dryland or acid wetland, both of which make plants experience abiotic stress and further reduce crop production. Plant growth in acid dry-soils causes plants to experience high drought and aluminum (Al) stress (Ma et al. 2014; Kochian et al. 2015). In rice, it was reported that Al stress resulted in shorter root length, increased reactive oxygen species

(ROS) content, and inhibited plant growth (Fendiyanto et al. 2019a). Drought stress can also inhibit the expression of several drought tolerance regulatory genes in rice (Satrio et al. 2019). In wetlands, plants will also encounter Fe (Turhadi et al. 2018) and Al toxicity stress and will further result in a bronzing response, disrupted rice growth and development, and will further reduce production. Thus, it is important to look for varieties, gene sources, mechanisms, regulatory genes of rice plants that are tolerant of various abiotic stresses (drought, Al, and Fe toxicity). Therefore, this chapter aimed to understand how the rice tolerance mechanism to drought, Al, and Fe stresses (multi-abiotic stress) is based on multi-studies of genetics, genomics, molecular physiology, plant breeding, and their use to design new rice cultivars that are tolerant and adaptive to abiotic stresses by manipulating the genome using CRISPR/C as genome editing approach and omics-technology.

1.2 Genetic Improvement of Rice for Drought Stress Tolerance

1.2.1 Water Availability and Drought Stress Tolerance in Rice

Water availability is the most significant abiotic factor that influenced plant evolution. Plant growth and productivity are highly dependent on water availability, particularly in paddy rice. The terminology of drought condition from an agricultural perspective is defined as a period of less than average precipitation, less regular rainfall, or above-normal evaporation, often decreasing crop production (Nelson et al. 2014). The severity of the drought relies on several variables, i.e., frequency and distribution of rainfall, level of evaporation, and storage capacity of soil moisture (Farooq et al. 2009; Hayes et al. 2010).

Drought stress affects morphology, physiology, biochemistry, anatomy, and agronomical traits (Fig. 1.1). Drought is observed by a decrease in water status, leaf rolling, stomatal closure, and a decrease in growth (Anjum et al. 2011; Takahashi et al. 2020). By influencing multiple morpho-physiological and biochemical processes like photosynthesis, respiration, ion absorption, plant height, and nutrient metabolism, as well as phytohormones, drought decreases plant growth (Praba et al. 2009). Extreme drought can lead to photosynthesis disturbance and severe metabolism disruption, contributing eventually to plant death (Osakabe et al. 2014).

Plant vulnerability to drought, however, depends on the degree and length of stress, types of plant, and stages of development. As phenotypic markers, various drought-related characteristics, including root and shoot characteristics, osmotic adjustment capacities, water status, abscisic acid (ABA) quantity, and cell membrane stability, have been used to determine drought resistance (Barik et al. 2020). Drought tolerance mechanisms at the genetic and molecular levels have been intensively investigated in an attempt to the genetic improvement in rice (Vinod

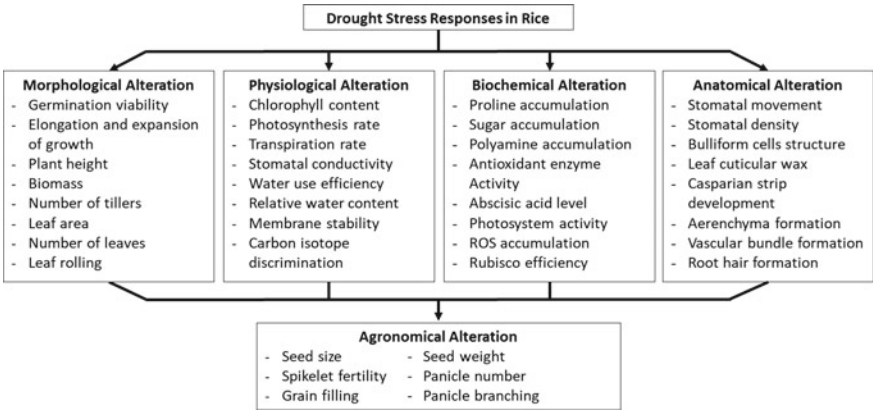

Fig. 1.1 Drought stress in rice induces various alteration that occurs at the morphological, physiological, biochemical, and anatomical levels, which in turn can affect the agronomicalalteration

et al. 2019). Drought stress responses are involved in various molecular networks (Todaka et al. 2015). Therefore, complete information of genes responsible for drought tolerance and dissection of their network is a key to understand drought stress tolerance and a way to conduct genomic designing for drought-stress resistance in rice.

1.2.2 Drought Tolerance Genes in Rice in Terms of Structural and Functional Genomics

Genes responsible for drought tolerance has been widely studied, either through structural and functional genomics approaches. Structural genomics refers to the localization and characterization of a set of genes responsible for a particular phenotype by physical or genetic mapping (Varshney et al. 2018). The structural genomics study on rice is growing rapidly along with the availability of the reference genome sequences for physical mapping (Kawahara et al. 2013). On the other hand, molecular markers for genetic mapping are also continuously developing. High-density genetic maps of 12 rice chromosomes on several genetic backgrounds are available (Harushima et al. 1998; Qu et al. 2020). The availability of these data that accompanied by the advanced phenotyping techniques and statistical methods causes genetic mapping is becoming increasingly easy to be performed. In contrast, functional genomics includes the use of both genomic and transcriptomic techniques to describe gene function that is specifically expressed for a particular phenotype (Li et al. 2018a, b). The study of expressed sequence tags is the earliest technique in the study of functional genomics (Satrio et al. 2019),

followed by microarray which is more efficient in studying multiple gene expression simultaneously and has now been replaced by the RNA sequencing technique (Byrne et al. 2019).

Several structural genomics studies based on genetic mapping have been performed to investigate the genes responsible for drought stress tolerance, using both family-based mapping or the natural population. Drought stress tolerance in rice is quantitatively inherited and regulated by many genes, called quantitative trait loci (QTLs) (Sinclair 2011; Sahebi et al. 2018). In the bi-parental mapping population, the identification of QTLs controlling drought tolerance traits includes a chain of activities such as the genotyping of populations using molecular markers; genetic maps construction; and separately, analyze the phenotypes of the population according to the drought tolerance traits; then perform QTL mapping regarding the results of genotyping and phenotyping steps (Barik et al. 2019). The natural population can also be performed to discover genes responsible for drought tolerance in rice. Linkage disequilibrium based on association mapping using the natural population can be performed using the steps that are similar to QTL mapping, with the addition of consideration to population structure and kinship (Wang et al. 2020a, b).

Several QTLs linked to phenotypes related to drought stress tolerance have been well studied in rice. However, only a few studies have been reported on yield, which is important for agronomic value. According to a wide range of important traits correlated to drought tolerance, including root and shoot alterations, phytohormonal responses, osmotic adjustment, photosynthesis, transpiration, and globally plant response to drought tolerance, many QTLs for drought tolerance correlated traits in rice varieties have been identified (Table 1.1).

Transcriptome profiling greatly facilitated the development of the drought-tolerance-related functional genomics. Numerous genes that were induced by drought stress have been identified using transcriptomic analysis systems, e.g., microarray and RNA sequencing. Several transcriptome-wide expression studies for drought response in rice have been conducted. A dozen pathways along with their hundreds of genes were induced under drought stress treatment in two contrasting drought-tolerant rice genotypes (Lenka et al. 2011; Muthurajan et al. 2018; Ereful et al. 2020). Drought responsive genes can easily be identified using this approach, but their role in drought tolerance has not been proven. Functional analysis is needed to characterize the mode of action of the genes in the drought tolerance related traits. The most well-known and comprehensive models related to the drought-induced gene expression network as a part of functional genomics are the Abscisic acid (ABA)-dependent signaling pathway and the ABA-independent regulatory network mediated through dehydration responsive element-binding (DREB) (Yamaguchi-Shinozaki and Shinozaki 2006).

ABA phytohormone is a molecule that promotes signal transduction during the response to drought stress (Raghavendra et al. 2010). The 9-cis-epoxycarotenoid dioxygenase (NCED) is a critical enzyme for ABA biosynthesis (Iuchi et al. 2001). The *NCED3* expression level has been increased among genes encoding *NCED* in rice under water deficit conditions (Weiner et al. 2010). The ABA intercellular transport mechanisms have also emerged to be essential for ABA-dependent

Table 1.1 Quantitative trait loci (QTLs) for drought tolerance correlated traits in rice

Trait	Number of QTLs	Chromosome	References
Biomass	28	1, 2, 3, 4, 6, 7, 8, 10, and 12	Prince et al. (2015), Saikumar et al. (2014), Mishra et al. (2013), Sellamuthu et al. (2011), Srividya et al. (2011), Vikram et al. (2011), Gomez et al. (2010), Bernier et al. (2007), Kumar et al. (2007), Lanceras et al. (2004)
Canopy temperature	8	1, 2, 3, 4, and 7	Prince et al. (2015), Saikumar et al. (2014), Gomez et al. (2010), Yue et al. (2008)
Drought index	15	2, 4, 7, 10, and 12	Prince et al. (2015), Sellamuthu et al. (2011), Gomez et al. (2010), Yue et al. (2008), Zheng et al. (2008), Bernier et al. (2007), Li et al. (2005), Hemamalini et al. (2000)
Flowering time	8	2, 3, and 6	Prince et al. (2015), Palanog et al. (2014), Saikumar et al. (2014), Sandhu et al. (2017)
Grain weight	13	1, 2, 3, 5, 11, and 12	Prince et al. (2015), Zhou et al. (2013), Srividya et al. (2011), Zou et al. (2005), Thomson et al. (2003), Moncada et al. (2001)
Grain yield	74	All chromosomes	Prince et al. (2015), Palanog et al. (2014), Saikumar et al. (2014), Sandhu et al. (2017), Verma et al. (2014), Mishra et al. (2013), Zhou et al. (2013), Dixit et al. (2012), Ghimire et al. (2012), Sellamuthu et al. (2011), Srividya et al. (2011), Vikram et al. (2011), Bernier et al. (2007), Kumar et al. (2007), Xu et al. (2005), Lanceras et al. (2004)
Panicle length	7	1, 2, 3, 4, 8, and 11	Prince et al. (2015), Sellamuthu et al. (2011), Thomson et al. (2003), Lafitte et al. (2002)
Panicle number	9	1, 3, 4, 5, and 6	Mishra et al. (2013), Sellamuthu et al. (2011), Zou et al. (2005), Lafitte et al. (2004), Thomson et al. (2003), Lafitte et al. (2002), Moncada et al. (2001)
Seed setting rate	21	All chromosomes, except 11	Prince et al. (2015), Zhou et al. (2013), Sellamuthu et al. (2011), Srividya et al. (2011), Gomez et al. (2010), Thomson et al. (2003)
Tiller number	1	4	Hemamalini et al. (2000)
Plant height	27	1, 2, 3, 5, 7, 8, 9, 11, and 12	Prince et al. (2015), Saikumar et al. (2014), Sandhu et al. (2014), Mishra et al. (2013), Ghimire et al. (2012), Sellamuthu et al. (2011), Srividya et al. (2011), Vikram et al. (2011), Gomez et al. (2010), Lafitte et al. (2002), Venuprasad et al. (2002)
Root thickness	13	1, 2, 3, 4, 6, 7, 8, 9, and 12	Zhang et al. (2001), Ali et al. (2000)

(continued)

Table 1.1 (continued)

Trait	Number of QTLs	Chromosome	References
Root weight	8	1, 2, 4, 6, 9, 10, and 12	Zhang et al. (2001), Ali et al. (2000)
Root length	5	3, 4, and 12	Zhang et al. (2001), Ali et al. (2000)
Cellular membrane stability	9	1, 3, 7, 8, 9, 11, and 12	Tripathy et al. (2000)
Relative water content	4	1, 2, 5, and 9	Khowaja et al. (2009)
Osmotic adjustment	10	1, 2, 3, 7, 8, and 9	Zhang et al. (2001), Lilley et al. (1996)

drought responses (Cutler et al. 2010). ABA Phytohormone is synthesized by particular rice cells in vascular tissue and transferred to target cells, i.e., guard cells (Bauer et al. 2013; Kuromori et al. 2014). Protein receptor complex consisting of pyrabactin resistance (PYR)/PYR1-like(PYL)/a regulatory component of aba response (RCAR), protein phosphatase 2C (PP2C), and sucrose non-fermenting-1-related protein kinase 2 (SnRK2) is perceived by ABA (Kim et al. 2012; Miyakawa et al. 2013). The PP2Cs inhibit the ABA signaling via the inactivation of SnRK2s in the absence of ABA. The ABA-bound PYL/PYR/RCARs recognize and interact with PP2Cs in the presence of ABA, thus generating SnRK2s from PP2C-dependent negative control. The SnRKs activate downstream proteins was similar to transcription factors of ABA-responsive cis-element binding protein/ABA-responsive binding factor of cis-elements (AREB/ABF). In the AREB/ABF transcription factors, a bZIP domain, and four conserved domains containing SnRK2 phosphorylation sites have been identified (Umezawa et al. 2010). The phosphorylated AREB/ABFs are triggered and bind to the enriched ABA-responsive cis-element in drought-inducible gene promoter regions (Behnam et al. 2013). Under drought stress conditions, AREB/ABFs serve as major transcriptional activators controlling the expression of ABRE-dependent gene, particularly in ABA signaling.

The ABA-independent signaling pathway is also essential in rice response to abiotic stress (Raghavendra et al. 2010; Mizoi et al. 2012). The DREB2 proteins are members of the plant-specific transcription factors family of AP2/ERF (Qin et al. 2008). *DREB2A* and *DREB2B* genes are specifically induced by drought stress among the other *DREB2* genes and the protein serves as transcriptional activators through an ABA-independent pathway (Sakuma et al. 2006a, b). Under control conditions (without drought stress), the degradation of DREB2A via DRIP1 (DREB2A-interacting protein1) and DRIP2, the proteins with C3HC4 ring domain, occur through ubiquitination of DREB2A (Morimoto et al. 2013).

Structural and functional genomics studies have respective advantages and disadvantages; thus, it should be interesting if the two approaches are combined. Recently, the studies that combine both approaches are still limited. The structural genomics research is generally carried out by genetic mapping using the segregation population, then followed by fine-mapping using backcross or near-isogenic lines population for narrowing-down the responsible genes for the particular traits. Marker-assisted breeding is then being used to apply these genes for genetic improvement in rice plants (Muthu et al. 2020). In contrast with functional genomics research, which performs transcriptome expression analysis on two genotypes that have particular contrasting phenotypes, including the use of mutants. The gene candidates detected based on this information were characterized by overexpression- or suppression-based functional analysis. The genes that have been characterized could then be used for genetic improvement through transgenesis or genome-editing technique (Kasuga et al. 1999; Usman et al. 2017; Kumar et al. 2020).

We have applied a combination of structural and functional genomics approaches to examine and characterize candidate genes for drought tolerance in rice. We conducted QTL mapping on RILs population derived from parents that had contrasting phenotypes when treated under drought-stress conditions. Hawara Bunar is an Indonesian landrace that is well adapted to the upland environment (Satrio et al. 2019; Miftahudin et al. 2020), along with IR64 which is sensitive to drought stress, were used as the RIL parental lines. We carried out the genetic mapping at two developmental stages, i.e., vegetative and reproductive, which was conducted in the greenhouses and field, respectively. The genetic mapping successfully identified several regions of conservative QTL in two studies. Differential gene expression (DGE) using a meta-analysis technique was then applied to genes within the QTL region. A total of 14 candidate genes responsible for drought tolerance were obtained from a combination of structural and functional genomics approaches (Table 1.2).

1.2.3 Genes Responsible for Increasing Drought Tolerance in Rice

By expressing different types of protein that include transcription factors, chaperones, enzymes, and other functional proteins, plants have formed stable pathways or signaling chain processes to deal with drought stress (Maruyama et al. 2014; Todaka et al. 2015). Those proteins increase the resistance of plants to conditions of drought (Oladosu et al. 2019). Using structural and functional genomic approaches, hundreds, or even thousands of regulatory and functional genes have been identified (Varshney et al. 2018). Specifically to rice, the several genes have been introduced into the rice to investigate their effect especially on drought treatment (Table 1.3).

Table 1.2 Genes within QTL for drought tolerance traits in rice related to intercellular CO_2, stomatal conductance, net photosynthetic rate, transpiration efficiency, and water use efficiency in the vegetative and reproductive stage, that differentially expressed based on the transcriptomes meta-analysis

Gene name	LFC	p-value	Description
OsSUI1	−0.15	2.0E −03	Translation initiation factor SUI1 domain containing protein
OsbHLH25	−1.16	1.0E −02	Basic helix-loop-helix (bHLH) transcription factor, diterpenoid phytoalexin factor, biosynthesis of diterpenoid phytoalexins, stress response
OsRH20	−0.23	5.3E −05	DEAD-box ATP-dependent RNA helicase 20
OsRDR3	0.86	1.0E −03	RNA-dependent RNA polymerase, eukaryotic-type domain-containing protein
OsRDR4	1.48	1.7E −15	RNA-dependent RNA polymerase, eukaryotic-type domain-containing protein
OsCK	0.12	2.9E −03	Similar to casein kinase-like protein
OsABH	1.13	3.1E −16	Alpha/beta hydrolase family protein
OsAIRC	−0.66	4.1E −16	Similar to phosphoribosylaminoimidazole carboxylase catalytic subunit
OsATG3A	0.44	2.1E −06	Similar to autophagocytosis protein AUT1-like
OsHOX29	2.00	5.2E −19	Similar to homeobox-leucine zipper protein HOX29
OsMPPN	−0.40	3.1E −05	Mitotic phosphoprotein N'endfamily protein
OsERF39	2.85	9.6E −10	Similar to Avr9/Cf-9 rapidly elicited protein 111A
OsOXT	−0.80	2.3E −03	Similar to xylosyltransferase
OsMADS3	1.04	5.8E −04	Transcription factor, floral organ development, lodicule development, stamen specification, floral meristem determinacy, male reproductive development

1.2.3.1 Genes Encoding Proteins for Osmotic Adjustment

Accumulation of osmolytes may protect the structural integrity of membranes. Several previous studies have indicated that rice was more tolerant to water stress that favored by osmotic modification. Osmotic tolerance is believed to be one of the mechanisms for escaping water stress (Ahmed et al. 2009). Under varying adverse conditions, proline functions as an osmotic adjustment in rice. Differences in normal proline concentration and stress conditions in rice have been identified. In addition to this study, under drought stress, proline exhibits three functions, i.e., a

Table 1.3 Genes responsible for drought-stress tolerance that has been introduced in rice

Gene	Gene description	Phenotype alteration	References
ADC	Arginine decarboxylase (polyamine biosynthesis)	Inhibit chlorophyll loss under drought stress; improved water-deficit tolerance via secreting higher levels of polyamines synthesis	Capell et al. (1998, 2004)
PYL/ RCAR5	ABA receptor	Perceived ABA in ABA-dependent signaling pathway and increase drought stress tolerance	Kim et al. (2014)
DSM2	Abscisic acid metabolism	Improve oxidative and water-deficit tolerance; increase xanthophylls contents, and; modulated non-photochemical quenching in photosynthesis	Du et al. (2010)
OAT	Amino acid metabolism	Increase drought tolerance and seed setting via amino acid metabolisms	You et al. (2012)
SRO1c	Reactive oxygen species scavenging	Regulates oxidative stress tolerance by scavenging ROS and regulates stomatal closure in leaves	You et al. (2013)
PPO	Protoporphyrinogen oxidase	Decrease in oxidative damage, and improve drought tolerance	Phung et al. (2011)
TPSP	Trehalose synthesis	Has roles in many abiotic stresses especially drought, cold, and high salinity in rice seedling developmental stage; cold, salt, and drought tolerance was modulated via chlorophyll fluorescence mechanisms	Li et al. (2011), Jang et al. (2003)
P5CS	Proline synthesis	Tolerance to several abiotic stresses, i.e., drought and salinity stress; increased yields under salinity and drought stresses	Su and Wu (2004), Zhu et al. (1998)
HVA1	Late embryogenesis abundant	Has roles as cell membrane stability, improve higher leaf relative water content (RWC) and increase in	Babu et al. (2004a, b), Rohila et al. (2002), Xu et al. (1996)

(continued)

Table 1.3 (continued)

Gene	Gene description	Phenotype alteration	References
		growth; drought and salinity tolerance; Increased drought tolerance, and salinity stress	
LEA3	Late embryogenesis abundant	Increased yield under drought condition; drought resistance and increase grain per panicle	Duan and Cai (2012), Xiao et al. (2007)
ABF3	ABF transcription factor	Regulated many genes under salinity and drought stresses	Oh et al. (2005)
AP37	AP/ERF transcription factor	Increase morpho-physiologically growth performance under drought condition	Oh et al. (2009)
bHLHU8	bHLH transcription factor	Drought resistance and ABA sensitivity	Seo et al. (2011)
bZIP16	bZIP transcription factor	Drought stress tolerance	Chen et al. (2012a, b)
bZIP23	bZIP transcription factor	Increase tolerance to salt and drought stresses in broad-spectrum and improvement in grain yield	Xiang et al. (2008)
bZIP46	bZIP transcription factor	Drought resistance and hypersensitive by exogenous ABA	Tang et al. (2012)
bZIP71	bZIP transcription factor	Tolerance to drought stress	Liu et al. (2014)
bZIP72	bZIP transcription factor	Drought resistance and ABA sensitivity	Lu et al. (2009)
DERF1	AP2/ERF transcription factor	Increase drought tolerance	Wan et al. (2011)
ERF4a	AP2/ERF transcription factor	Increase drought tolerance	Joo et al. (2013)
ERF10a	AP2/ERF transcription factor	Increase drought tolerance	Joo et al. (2013)
TSRF1	AP2/ERF transcription factor	Enhances the osmotic and drought tolerance	Quan et al. (2010)
JERF1	AP2/ERF transcription factor	Drought tolerance	Zhang et al. (2010a)
JERF3	AP2/ERF transcription factor	Increase drought stress tolerance	Zhang et al. (2010b)
DREB1			

(continued)

Table 1.3 (continued)

Gene	Gene description	Phenotype alteration	References
	AP2/ERF transcription factor	Regulates many genes correlated to water-deficient, low-temperature, and high-salt stresses	Oh et al. (2005), Ito et al. (2006), Xiao et al. (2009), Datta et al. (2012), Ishizaki et al. (2012)
DREB2	AP2/ERF transcription factor	Improve grain yield under drought stress	Bihani et al. (2011), Cui et al. (2011)
CBF4	AP2/ERF transcription factor	Tolerance to drought, high-salinity, and low-temperature	Oh et al. (2007)
HARDY	AP2/ERF transcription factor	Increase drought tolerance	Karaba et al. (2007)
MYB2	MYB transcription factor	Increase drought tolerance	Yang et al. (2012)
NAC5	NAC transcription factor	Increase drought tolerance	Jeong et al. (2013)
NAC6	NAC transcription factor	Increase drought tolerance	Nakashima et al. (2007)
NAC9	NAC transcription factor	Increase drought tolerance	Redillas et al. (2012)
NAC10	NAC transcription factor	Increase drought tolerance	Jeong et al. (2010)
SNAC1	NAC transcription factor	Increase drought tolerance	Hu et al. (2006)
WRKY30	WRKY transcription factor	Increase drought tolerance	Shen et al. (2012)
ZFP182	Zinc finger transcription factor	Increase drought tolerance	Huang et al. (2012a, b)
ZFP245	Zinc finger transcription factor	Increase drought tolerance	Huang et al. (2009a, b, c)
ZFP252	Zinc finger transcription factor	Increase drought tolerance	Xu et al. (2008)
ZAT10	Zinc finger transcription factor	Increase drought tolerance	Xiao et al. (2009)
CPK4	Calcium-dependent protein kinase	Increase drought tolerance via a kinase signaling cascade	Campo et al. (2014)
CDPK1	Calcium-dependent protein kinase	Increase drought stress tolerance via a kinase signaling cascade	Ho et al. (2013)
CDPK7	Calcium-dependent protein kinase	Increase drought stress tolerance via a kinase signaling cascade	Saijo et al. (2000)
CDPK12	Calcium-dependent protein kinase		Xiang et al. (2007)

(continued)

Table 1.3 (continued)

Gene	Gene description	Phenotype alteration	References
		Increase drought tolerance via a kinase signaling cascade	
NPK1	MAP kinase	Increases in grain yield under drought stress	Xiao et al. (2009)
HRF1	Harpin protein	Drought resistance via ABA signaling and antioxidants, and acts in stomatal closure regulation	Zhang et al. (2011)
COIN	RING finger protein	Cold, salt, and drought stress tolerance	Liu et al. (2007)
SAP8	Stress/zinc finger protein	Acts in salt, drought, and cold tolerance	Kanneganti and Gupta (2008)
RDCP1	Protein degradation	Improved drought tolerance	Bae et al. (2011)
SDIR1	Protein degradation	Stomata regulation under drought conditions	Gao et al. (2011)

signaling molecule, an antioxidative defensive molecule, and an osmotic adjustor (Liang et al. 2013; Fahramand et al. 2014). Increasing the rate of antioxidant activity by the accumulation of proline could prevent cellular damage. Furthermore, proline is also known as a scavenger of reactive oxygen species and avoids oxidative damage. In order to sustain osmotic sensitivity, plants exposed to drought stress raise their proline (Lum et al. 2014). The level of proline accumulation has been documented to be depending on the degree of water deprivation and the species of plants (Koskeroglu and Tuna 2010). The proline content can thus be used as a marker to differentiate drought resistance in rice. The overexpression of the proline biosynthesis gene *P5CS* in rice plants shows a great increase in drought tolerance (Zhu et al. 1998). Similarly, the overexpression of the *OsOAT* gene can increase the proline level and strengthened the resistance to drought stress (You et al. 2012).

Trehalose plays an important role in abiotic stress such as drought, also known as tremalose or mycose. The trehalose stabilizes proteins against denaturation and defends against stress. Trehalose accumulation in rice has been documented to increase the resistance to drought. When a *TPP/TPS* gene for trehalose biosynthesis was introduced into rice plants, the results revealed an improvement in the resistance of drought by reducing the level of photooxidation (Jang et al. 2003).

1.2.3.2 Genes Encoding Proteins for Late Embryogenesis Abundant

The late embryogenesis abundant (LEA) proteins are mostly present in plants and involve a variety of proteins that are fundamentally unorganized (Yadira et al. 2011). During the maturation of the embryo, these small proteins are produced and act as chaperones, ranging from 10 to 30 kDa (Duan and Cai 2012). A cellular water deficit generated by drought contributes to the aggregation of LEA proteins. Several studies in plants revealed that overexpression of LEA proteins from different classes confers tolerance for a range of treatments for water deficit. In response to water deficit stress, OsLEA3 overexpression in rice plants increased drought tolerance (Xiao et al. 2007). When the gene encoding the LEA protein, HVA1, from barley was over-expressed in rice, there was a significant increase in growth and water-use capacity under drought stress conditions (Sivamani et al. 2000; Babu et al. 2004a, b). The gene encoding LEA protein, *OSLEA3-1* was investigated to play an important role in the regulation of drought stress in rice (Xiao et al. 2007). The *OsLEA3-2* overexpression in rice has also demonstrated a strong drought-tolerance phenotype, with a lower yield loss relative to control under drought-field conditions (Duan and Cai 2012).

1.2.3.3 Genes Encoding Proteins for Signal Transduction

Overexpressing *OsCPK4*, a calcium-dependent protein kinase in rice plants demonstrated increased resistance to drought stress. The genes associated with lipid metabolism were up-regulated in the transgenic plants, such as those encoding proteins with lipid binding activities, lipid transport proteins, and lipases. The transgenic plants were also more expressing oxidative stress-responsive genes (Campo et al. 2014). These results indicate that *OsCPK4* is involved in controlling the defense of cellular membranes against oxidative stress. Transgenic rice plants overexpressing another kinase gene, *OsCDPK1* have also been generated. Enhanced drought resistance and enabled the expression of a gene for a *GF14c* protein were seen in transgenic rice plants (Campo et al. 2012). Transgenic rice plants overexpressing *GF14c* have also shown enhanced drought tolerance, meaning that *GF14c* can mediate increased drought tolerance due to *OsCDPK1* (Ho et al. 2013). Another kinase, *OsCIPK12* was also involved in enhancing drought-stress tolerance responses by modulating levels of proline and dissolved sugars in cells (Saijo et al. 2000; Xiang et al. 2007).

1.2.3.4 Genes Encoding Proteins for the Transcription Factor

The biggest transcription factor family involved in the drought-tolerance response is bZIP (Nijhawan et al. 2008). The *OsbZIP23* is a crucial regulator of ABA-dependent pathways of gene expression network for drought tolerance (Kang et al. 2002). At both the germination and post-germination stages, the rice plants

overexpressing *OsbZIP23* showed improved ABA sensitivity and demonstrated increased tolerance to drought stress. Hundreds of *OsbZIP23* downstream genes with various roles, such as transcription factors, protein kinases, dehydrins, and LEA proteins have been identified by transcriptomics technique (Xiang et al. 2008). *OsbZIP46* seems to have a similar role as *OsbZIP23*. Increased drought resistance was demonstrated by transgenic rice plants overexpressing *OsbZIP46*. The transcriptome technique was then used to identify up-or down-regulated genes regulated by *OsbZIP46* in transgenic rice plants. These differentially controlled genes varied greatly from the downstream genes of *OsbZIP23*, meaning that *OsbZIP46* controls a different group of genes than *OsbZIP23* does (Tang et al. 2012). The other bZIP members, *OsbZIP16* and *OsbZIP71*, were also involved in enhancing the resistance of drought in rice. Transgenic rice overexpressing *OsbZIP16* and *OsbZIP71* showed a higher drought tolerance level than its wild type. Transgenic rice overexpressing *OsbZIP16* and *OsbZIP71* demonstrate increased resistance to drought (Chen et al. 2012a, b; Liu et al. 2014). These studies indicate that the transcription factors of the bZIP type involved in the ABA-signaling pathway are potentially useful for developing rice varieties with improved drought tolerance. This opinion is reinforced by the fact that drought stress raises the ABA content in rice (Cutler et al. 2010).

The *DREB1A* is an AP2/ERF-type transcription factor that plays a key role in the drought-stress response regulator (Chen et al. 2008; Datta et al. 2012). In rice plants, overexpression of *DREB1A* regulated by the ubiquitin promoter increases drought resistance (Oh et al. 2005; Ito et al. 2006). In contrast to that of non-transgenic plants, transgenic rice plants expressing DREB1A under the influence of the ubiquitin or stress-inducible promoter *RD29* showed an improvement in yield under conditions of drought stress (Wan et al. 2011). Elevated osmo protective content was also found in the transgenic plants, such as free proline and soluble sugars. In transgenic rice plants, a transcriptome technique has detected up-regulated genes, particularly alpha-amylase, dehydrin genes, and other stress-responsive genes (Ishizaki et al. 2012). The *DREB2A* biochemical involvement and molecular structure in abiotic stress responses have been thoroughly investigated. As a master regulator of the drought stress response, *DREB2A* is commonly recognized and has a high potential to increase tolerance to drought stress (Matsukura et al. 2010). In different plant species, the *DREB2* regulation system appears to be well conserved. It has been observed that transgenic rice plants overexpressing *OsDREB2B* improve drought tolerance. Under the control of an ABA-responsive promoter, overexpression of OsDREB2A in rice can induce soluble sugar and proline content in the seedling, resulting in enhanced osmotic stress tolerance in rice (Cui et al. 2011; Maruyama et al. 2012).

In addition to subfamily members of *DREB1A* and *DREB2*, multiple AP2/ERF-type transcription factors have been performed to generate transgenic rice with improved resistance to drought stress (Mizoi et al. 2012). The *HARDY* gene is a transcription factor similar to AP2/ERF that increases the tolerance of drought stress (Karaba et al. 2007). Overexpressing *HARDY*, transgenic rice plants exhibit better photosynthesis and transpiration performance, which leads to shoot and root

biomass improvement. *OsDERF1* is an upstream regulator of *OsERF3* and *OsAP2-39* (Zhang et al. 2013). The *OsDERF1* knockdown improves ethylene biosynthesis and modulates drought response. The overexpression of *OsERF3*/*OsERF4a*, by contrast, reduces the level of expression of a transcriptional repressor induced in defensive responses, contributing to improved resistance to drought stress (Joo et al. 2013). Overexpression of *OsJERF3* and *TSRF1* in independent transgenic rice plants exhibits increase drought resistance (Quan et al. 2010; Zhang et al. 2010a). Corresponding changes in the expression of genes encoding *MYC*/*MYB* transcription factors and genes related to ABA and proline biosynthesis and photosynthesis are linked to the drought improvement. In contrast with those of non-transgenic plants, the overexpressed rice showed higher levels of proline and soluble sugar contents. Increased resistance to drought stress was demonstrated by transgenic rice plants over-expressing *AP37*, a rice AP2/ERF transcription factor. The transcriptome technique identified the AP37 downstream genes, which included iron transporter and PHD zinc finger transcription factor genes (Oh et al. 2009).

The NAC family transcription factors act in a broad range of development and drought stress tolerances (Nakashima et al. 2012; Nuruzzaman et al. 2013). To improve drought stress tolerance, the constitutive overexpression of *OsNAC6* in rice plants has been generated. Transcriptomic research in the study reported several *OsNAC6* downstream genes, including drought-responsive genes (Nakashima et al. 2007). The *SNAC1* was shown to be predominantly expressed under conditions of drought in guard cells. Overexpression of *SNAC1* in rice plants showed decreased dehydration due to improved stomatal closure and expression level of stress-related genes (Hu et al. 2006). Under the influence of a root-specific promoter in conditions of drought stress, independent transgenic plants overexpressing *OsNAC10*, *OsNAC5*, and *OsNAC9/SNAC1* recorded thicker roots and higher agronomic yields than those of control plants (Jeong et al. 2010; Redillas et al. 2012). Proline and soluble sugar accumulations were higher, as well as root diameter was increased in *OsNAC5* overexpressors than in non-transgenic plants (Song et al. 2011; Jeong et al. 2013). The *OsNAC5* acts as a transcriptional initiator and controls the transcript level of several stress-responsive genes (Takasaki et al. 2010). To increase drought resistance, the *OsWRKY30* over expressor had also been generated. The observed association with different MAP kinase proteins of these *OsWRKY30* proteins shows that *OsWRKY30* acts downstream of the MAPK cascades (Shen et al. 2012). Some studies have stated that in transgenic rice, the C2H2-type zinc finger overexpression enhances water-deficit tolerance.

Several drought tolerance genes demonstrated an ability to improve rice tolerance to drought stress in the field trials (Table 1.3). Thus, it can further be applied to rice cultivation in the dry land or season. Genes that have been well characterized for their function in increasing drought tolerance can also be applied to breeding drought-tolerant rice plants, yet field trial is needed. Furthermore, candidate genes responsible for drought tolerance which are mainly identified by structural and functional genomics approaches have the potential as a resource to contribute to increasing drought-stress resistance in rice through genomic designing.

1.3 Genetic Improvement of Rice for Aluminum and Acid Tolerance

1.3.1 Acid Soil and Aluminum Problem in Rice Cultivation

Aluminum (Al) is one of the main limiting factors of plant growth in acid soils, including rice growth. Based on its prevalence, acid soils are found in the world in both tropical and temperate regions, reaching ~30% of the world's area and covering ~12% of arable crops area (von Uexkuell and Mutert 1995). Yellow Redpodsolic acid soil contains dissolved aluminum (Al) in the form of Al^{3+} that toxic for plant roots (Kochian 1995). In addition, soil acidity level has increased sharply due to excessive use of N fertilizers, acid rain, mono-cultivation, and modern-intensive agriculture (Guo et al. 2010; Yang et al. 2013; Long et al. 2017). After oxygen (O) and silicon (Si), aluminum was ranked 3rd for the amount of the most abundant element in the earth's crust and reached 7% of its mass (Foy et al. 1978). In neutral or mildly acidic soils, Al is dominantly inactive. Conversely, in acid soils (pH < 5), such as yellow-redpodsolic soils that tend to have a soil pH below 5, Al is in a dissolved form, Al^{3+} (Kinraide 1991) that can be toxic to plants, stop mitosis, inhibit cell division, inhibit root elongation, and damage root caps (Kochian 1995; Matsumoto 2000; Ma et al. 2014; Kochian et al. 2015). Consequently, the root system can be damaged causing the plants susceptible to drought and nutrient deficiency, hence inhibit plant growth and decrease yield (Kochian et al. 2004; Kochian 1995; Guo et al. 2018). Thus, Al toxicity is a major constraint for crop productivity worldwide including rice (von Uexkuell and Mutert 1995; Kochian et al. 2004; Reyes-Díaz et al. 2015; Chen et al. 2010; Guo et al. 2018). When rice roots are exposed to Al stress, the rice roots will be stunted and damage, which consequently the nutrient and water absorption area of the roots will be disrupted and the yield will decrease (Ismunadji and Partohardjono 1985; Matsumoto 2000; Ma et al. 2014; Mossor-Pietraszewska 2001; Kochian et al. 2004; Lynch and Saint-Clair 2004; Kochian et al. 2005).

1.3.2 Physiological Studies of Aluminum Tolerance in Gramineae Family Including Rice

Cereal crops (Gramineae) have been the main focus of research on Al-tolerance (Kochian et al. 2005). Al tolerance levels in the Gramineae family are widely dispersed both within and among species (Foy et al. 1978; Sasaki et al. 2006; Pineros et al. 2005; Furukawa et al. 2007; Caniato et al. 2007; Famoso et al. 2010). Among the major cereal species that have been studied extensively (rice, maize, wheat, barley, and sorghum), rice exhibits superior levels of Al-tolerance both in the field and hydroponic conditions (Foy et al. 1978; Famoso et al. 2010). Although rice is 6–10 times more Al-tolerant than other cereals, very little is known about the

genes underlying this tolerance. Based on its high level of Al tolerance and many genetic and genomic sources, rice provides good information or models for studying genetics, genomics, and morpho-physiology of Al tolerance.

In wheat, sorghum, and barley, the Al tolerance mechanism is inherited as a simple trait, controlled by one or more genes (Sasaki et al. 2004; Magalhaes et al. 2004; Minella and Sorrells 1992). However, in maize, rice, and *Arabidopsis*, Al tolerance is inherited quantitatively (Ninamango-Cardenas et al. 2003; Nguyen et al. 2001). Several Al tolerance genes have been cloned from wheat and sorghum. Wheat Al-tolerance gene, *ALMT1*, encodes the Al-activated malate transporter (Sasaki et al. 2004), while the Al-tolerance gene in sorghum, *SbMATE*, encodes Al-activated citrate efflux transporter in roots (Magalhaes et al. 2007; Hoekenga et al. 2003; Nguyen et al. 2002).

Li et al. (2000) stated that the organic acids secreted from plant roots as a response to Al stress are different among plant species. Tobacco and rice, as well as wheat and buckwheat, will immediately secrete organic acids as soon as they are exposed to Al (Ryan et al. 1995; Delhaize et al. 2001; Zheng et al. 2005; Ratnasari et al. 2016). In rye (*Secale cereale* L.) secretion of organic acids occurred several hours after Al stress (Li et al. 2000). Malic acid secretion in wheat is not inhibited by low temperature but it causes inhibition of citric acid secretion in the rye.

Physiologically, Al does not induce enzymes involved in the synthesis and metabolism of organic acids, but it does induce transport proteins for certain organic acids (Delhaize et al. 1993; Ryan et al. 1995). Al stress tolerance mechanisms involving the removal of organic anions such as malate and citrate from the root tip are controlled by genes from the ALMT gene family (Sasaki et al. 2004) and MATE (Magalhaes et al. 2007). This family of genes encodes membrane proteins, which are transporters in the membrane that help excrete malate and citrate across the plasma membrane.

1.3.3 General Mechanism of Plants and the Exclusion-Specific Mechanism of Rice in Detoxifying Aluminum

Several plants showed different responses to Al stress. Plant tolerance mechanisms to Al stress are grouped into 2 strategies, e.g., inclusion/Al-tolerance and exclusion. The inclusion or tolerance mechanism is a plant mechanism by allowing trivalent Al to enter the cells and simultaneously continue to detoxify it through the formation of Al complexes in certain organelles, therefore, it will not harmful to plants (Ryan et al. 2011). In other words, Al tolerance/inclusion is a plant protection mechanism against Al stress in which Al enters the root cells, is collected and detoxified in the subcellular compartment, and/or is translocated away from the root tip (Kochian et al. 2015). Inclusion is mechanisms that accommodating Al in vacuoles, such as in *Melastoma*, or accumulating Al in the shoot and roots, such as

in *Hydrangea*, wheat (Buckwheat), *Melastoma malabathricum*, and tea (*Camellia sinensis*). Al accumulation in plants can occur in the roots (Delhaize et al. 1993) and the canopy (Watanabe and Osaki 2001). Plants retain and accumulate Al in the roots, especially in the cortex and epidermis of the roots. In young tissue that does not yet have an epidermis, Al can escape into the shoot through the root vessels. In wheat plants, if Al has exceeded the threshold that can be tolerated by the cytoplasm, the Al accumulated in the roots will be excreted. The protein involved in the secretion of Al from wheat roots is controlled by the *Alt1* gene (Delhaize et al. 1993). There are also plants called Al accumulators, which are plants capable of accumulating Al in the shoot, such as *Melastoma malabathricum* L., tea (*Camellia sinensis*), and mangroves (*Rhizophora mangle*) (Watanabe and Osaki 2001). The tolerance mechanism in rice is carried out by the transporter protein natural resistance-associated macrophage protein (Nramp) Al transporter 1 (OsNrat1) to enter Al into cells, then insert and accommodate Al into vacuoles through the vacuolar transporter protein, particularly. *O. sativa*-ABC Al-sensitive 1 (*OsALS1*) (Huang et al. 2009a, b, c; Li et al. 2014; Xia et al. 2010, 2011, 2014). However, Ma et al. (2014) reported that the major Al mechanism in rice is exclusion by secreting organic acid.

Al exclusion is a plant protection mechanism against Al stress by involving organic acids or phenolic compounds to chelate Al in the rhizosphere (Kochian et al. 2015). The exudation of negatively charged organic acid anions into the rhizosphere can chelate Al^{3+} to form a nontoxic form and prevent or reduce Al from entering the roots. Some of the organic ions, i.e., malate, citrate, and oxalate, are released by the roots for this strategy include (Li et al. 2000; Ryan et al. 2011). Apart from organic acids, several plant species, such as phenolic compounds, also releases into the rhizosphere to chelate Al (Kochian et al. 2015).

Physiological evaluation of Al response on *Phaseolus vulgaris* and wheat showed both species are resistant to Al by increasing Al exudation and chelation by organic acids, such as citrate and malate (Delhaize et al. 1993; Miyasaka et al. 1991). A gene encoding a malate and citrate transporter has been found in the roots of wheat, sorghum (*Sorghum bicolor*), and barley (*Hordeum vulgare*) (Furukawa et al. 2007; Magalhaes et al. 2007; Sasaki et al. 2004). Two important families of transporter proteins include; the anion channel transporter the Al-activated malate transporter (ALMT) and the antiport transporter family OA/H^+ multidrug and toxic compound extrusion (MATE). Some plants, such as maize, secrete phenolic compounds (catechol, catechin, and quercetin) in addition to organic acid to chelate Al (Kidd et al. 2001).

1.3.4 Morpho-Physiological Parameters are Able to Describe Al-Tolerance Levels in Rice

Morpho-physiological characters of plants as a response to Al stress can be used as a parameter for Al tolerance. Among the morpho-physiological characters in the plant that can be used to describe the level of Al tolerance in rice are root-re growth (Roslim 2011), root length inhibition (Fendiyanto et al. 2019a), main root length (Fendiyanto et al. 2019a), total root length (Fendiyanto et al. 2019b), lateral roots length (Fendiyanto et al. 2019b), number of lateral roots (Fendiyanto et al. 2019b), malondialdehyde (MDA) content (Siska et al. 2017; Fendiyanto et al. 2019a), membrane lipid peroxidation (Siska et al. 2017; Fendiyanto et al. 2019a), and indirectly-chlorophyll content (Fendiyanto et al. 2019a).

Wijayanto (2013) and Fendiyanto et al. (2019b) reported that the root length characters of the Al-stressed rice, i.e., total root length, main root length, lateral root length, and the number of the lateral root, could be a trait of QTL for Al tolerance that mapped on the rice chromosome 3 that linked to markers RM545, SNPB11, and RM14543. The number of lateral roots and main root length showed additive and epistatic gene actions, respectively (Fendiyanto et al. 2019b). The mode of action of the epistatic gene was also found in the study of Wu et al. (2000). The epistatic gene action is the action of a gene that can mask the traits of other genes in QTL for Al tolerance, while the additive gene action has a phenotypic value from the sum of one gene and another in QTL. Wu et al. (2000) reported that Al tolerance in young seedlings was generally controlled by the additive effect of QTL, while in old seedlings was controlled by the epistatic effect of QTL. New findings obtained by Fendiyanto et al. (2019b) who reported that the main root length character, which has an epistatic effect, can be used as a good Al tolerance parameter for the rice to determine and select rice plants based on their level of tolerance to Al stress at early seedling stage (Fendiyanto et al. 2019b). Another root length of characters that can be used as an Al tolerance parameter in rice is the relative root length of the main root (Wijayanto 2013; Siska et al. 2017; Fendiyanto et al. 2019a). The character is determined by comparing the main root length of Al-stressed to the main root length of unstressed rice. As the Al-tolerance parameter, root length character will respond to the Al stress depending on the rice genotype and Al tolerance mechanism. Tolerant plants that have an exclusion mechanism can maintain root length during Al-stress by secreting organic acids into the rhizosphere and growing back after being restored (Kochian et al. 2015).

Physiological characters can also be used as Al tolerance parameters. Root histochemistry of Al accumulation, root membrane lipid peroxidation, and leaf chlorophyll content are among the physiological characters that are usually used in the Al tolerance analysis. Siska et al. (2017) reported that in addition to root growth, the histochemical test of Al accumulation in the root tips and lipid peroxidation of root cell membrane were used as Al tolerance parameters to distinguish the Al tolerance level between IR64 transgenic *OsGERLP* rice and its wild type.

The qualitative root histochemical test is a measure of Al accumulation in the root tip based on the intensity of purple color after staining the Al-stressed root with hematoxylin solution. The more intense the color, the higher the Al accumulation in the root. Rice cv. HawaraBunar showed lower intensity than cv IR64 and other Inpago rice after Al-stressed and staining with hematoxylin solution indicating that rice cv HawaraBunar is more tolerant to Al than that of other cultivar tested (Jumiati 2016; Siska et al. 2017; Fendiyanto et al. 2019a). HawaraBunar rice is Al tolerant cultivar and has an exclusion mechanism by secreting a high level of citric acid into the rhizosphere so that the Al content in root tip cells is relatively small.

Another physiological parameter of Al tolerance is a level of lipid peroxidation of the root cell membrane, which is represented by the concentration of malondialdehyde (MDA). The higher concentration of the MDA, the higher level of lipid peroxidation, the more sensitive the root of Al stress. Rice cv HawaraBunar showed lower MDA content compared to other Al-sensitive rice cultivars such as IR64 indicating that HawaraBunar is more tolerant of Al stress (Jumiati 2016; Siska et al. 2017; Fendiyanto et al. 2019a). The high concentration of MDA in the root tip cells indicates the root cell membrane experiencing membrane damage due to Al toxicity during/after being stressed with a certain level of Al.

Leaf chlorophyll content will decrease when plants are exposed to reactive oxygen species (ROS) that are produced when the plant roots are exposed to Al stress (Fendiyanto et al. 2019a). Although this character is the secondary symptom of Al toxicity, leaf chlorophyll content might be used as an Al tolerance parameter. Kochian et al. (2015) also reported that Al^{3+} content can be transported from roots to leaves and stored in vacuoles in leaf tissue in *Hydrangea* plants. The high Al content in the leaves can further damage the photosynthetic device in the leaves, thereby reducing the chlorophyll content. Al-sensitive Inpago rice cultivars have relatively lower chlorophyll content compared to Al-tolerant Inpago rice (Fendiyanto et al. 2019a). Rice cv HawaraBunar contains relatively high chlorophyll a, b and carotenoids compared to other upland rice cultivars (Fendiyanto et al. 2019a). However, since the chlorophyll content under Al stress is a secondary symptom due to the emergence of high ROS in cells that could be due to factors other than Al stress, the leaf chlorophyll content may not be an accurate Al tolerance parameter. Ma et al. (2014) observed that the parameter of plant tolerance to Al stress was root growth because the roots, especially the root tips, are the plant part that is first exposed to Al in the rhizosphere.

1.3.5 Genetic Study: QTL Mapping and GWAS Analysis of Al Tolerance in Rice

Genetic mapping of polygenic traits in plants is divided into two types, i.e., a quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS). The basis of QTL mapping to search for marker areas associated with a

specific phenotype is linkage recombination mapping, while GWAS uses linkage disequilibrium as the basis for association mapping (Ganal et al. 2012). QTL analysis can be done by creating an artificial population in the form of the recombinant inbred line (RIL), BC_1F_2 backcross population, or near-isogenic line (NIL), while GWAS analysis can be done using natural populations.

There are many studies related to QTL for Al tolerance in rice that hasbeen performed using various segregation population, genetic mapping techniques, and phenotypic analysis methods and have successfully identified QTL areas on various rice chromosomes (Ma and Furukawa 2003). Wu et al. (2000) reported that there were 8 QTLs for Al tolerance in the rice RIL population derived from a cross between var. Azucena × IR1552. They identified QTLs Al-tolerance trait located on rice chromosome 3 between markers ACC-CTG2, CDO1395, and AGC-CAG4 after the plant being Al-stressed for two weeks. In addition to the QTL analysis, a total of 12 interacted loci with significant epistatic effects have been found in Wu's studies. Furthermore, Wu et al. (2000) conducted a study to identify the genetic background of Al tolerance in rice particularly using nutrient solutions. In their study, a genetic linkage mapping was performed using 104 amplified fragment length polymorphism (AFLP) markers and 103 restriction fragment length polymorphism (RFLP) markers. Relative root length (RRL) character was measured on the rice RIL population after two and four weeks of stress treatment at a concentration of 1 mM Al^{3+} and control with pH 4. Two QTLs were detected at week 2 and 4 on chromosomes 1 and 12 with the QTL on chromosome 1 was only detected after 2 weeks of stress indicating the QTL on chromosome 1 is expressed in the early seedling stage. The effect of QTL on chromosome 12 increases with increasing the stress period from two to four weeks. They concluded that the level of Al tolerance in the young seedling was dominated by additive effect, while the late seedling phase was controlled by epistatic gene action (Wu et al. 2000).

One year later, Nguyen et al. (2001) identified 20 QTLs for Al tolerance in the F3 rice population derived from rice var. Chiembau X Omon 269-65. There was a specific QTL for Al tolerance based on root length and root length ratio and linked to markers RG996 and RZ142. Supporting the last study, a total of 10 QTLs for Al tolerance were identified scattered on different rice chromosomes of a segregation population originating from a cross between CT9993 × IR62266 (Nguyen et al. 2003). In another report, Nguyen et al. (2003) using different rice RIL populations (IR64 × *Oryza rufipogon*) found 5 QTLs for Al tolerance. Ma et al. (2002) using relative root elongation as an Al tolerance parameter successfully identified three QTLs for Al tolerance on chromosomes 1, 2, and 6 in 183 lines of RIL populations originating from Koshihikari (Al tolerant) and Kasalath (Al sensitive) crosses. Xue et al. (2006) identified 3 QTLs in the RIL population of Asominori × IR24. In addition to previous QTL studies, Xue et al. (2007) mapped three QTLs for Al tolerance on chromosomes 1, 9, and 11 in a RIL population derived from Asominori (Al-tolerant japonica) × IR24 (Al-sensitive indica) based on relative root elongation. Xia et al. (2014) identified about 1 QTL using Koshihikari × Kasalath substitution lines, while Zhang et al. (2016) identified 23 QTL in rice using 150 genotypes of landrace rice collections. Meng et al. (2015) found around

11 QTL in intercrossing population rice. Thus, several QTLs for Al tolerance have been reported in rice using 6 different inter-and intra-specific mapping populations with different genetic backgrounds, but using the similar Al-tolerance parameter, which is root length or root elongation character (Wu et al. 2000; Nguyen et al. 2001; Ma et al. 2002; Nguyen et al. 2003; Xue et al. 2006, 2007).

The last reported genetic studies particularly QTL for Al tolerance to potentially discover Al-tolerance genes in rice have been performed by Fendiyanto et al. (2019b). This finding was initiated by Miftahudin et al. (2007) who predicted that there was a QTL Al tolerance in the rice chromosome 3 region between simple sequence repeats (SSR) markers RM489 and RM517 using root re-growth (RRG), relative root length (RRL), and root length inhibition (RLI) as Al-tolerance parameters. This prediction was proven by Fendiyanto et al. (2019b) after constructing the linkage mapping using more SSR markers and an SNP marker SNPB11 derived from the *B11* allele-specific markers. The *SNPB11* marker was flanked by markers RM545 and RM14543 with the distance to both markers are 0.5 and 0.7 cM, respectively. Other markers that have been mapped in the same linkage group have relatively the same genetic distance as McCouch's (2002) study, i.e., RM489, RM2790, RM545, RM14543, and RM517 with the distance of 29.2, 30, 33.3, 34.5, and 42.9 cM, respectively. By employing two root growth characters, i.e., main root length and total root length, on 246 Al-stressed rice seedlings of an F9 RIL population derived from a cross between rice cv. IR64 × HawaraBunar, Fendiyanto et al. (2019b) identified a QTL for Al tolerance on rice chromosome 3 located in between markers RM545 and RM14543. The QTL was then remapped by Miftahudin et al. (2021) on the same chromosome by integrating marker SNPB11 with SNP markers developed by the genome base sequencing method on different rice lines from the same RIL population used by Fendiyanto et al. (2019b).

Another genetic mapping method to identify quantitative traits in the plant is a genome-wide association (GWAS) analysis. A rice genotype panel consisted of several rice varieties or lines is used as an object of the study to obtain genetic factors underlying certain traits in rice. The various rice genotype panels were reported to have similarities, or slightly higher levels of linkage disequilibrium (LD) compared to other species like *Arabidopsis*, maize, and humans. The mean of LD levels in rice hasbeen estimated to be between 50 and 500 kb (Garris et al. 2003; Olsen et al. 2006; Mather et al. 2007; Rakshit et al. 2007; McNally et al. 2009), depending on germplasm investigated, compared with 10–250 kb in *Arabidopsis* and humans (Daly et al. 2001; Jeffreys et al. 2001; Reich et al. 2001; Ching et al. 2002; Nordborg et al. 2002; Jung et al. 2004; Yu et al. 2006; Kim et al. 2007), about 100–500 kb in commercial elite in breds of maize, and 1–2 kb in maize on various landraces (Tenaillon et al. 2001; Remington et al. 2001). The inbreeding trait of rice, coupled with its demographic evolution, is a major determinant of the genome-wide LD pattern. Population sub-structure can lead to false positives in association mapping studies and must be taken into the analysis (Yu et al. 2005; Zhao et al. 2007; Atwell et al. 2010). The mixed linear model (MLM) method has been shown to work well on maize and *Arabidopsis* (Yu et al. 2005; Atwell et al. 2010) and this method has also demonstrated its ability to reduce

false-positive rates in rice when used in a single subpopulation (Huang et al. 2010). In addition, the population must also avoid the existence of false negatives to avoid bias in QTL mapping (Zhao et al. 2011).

As many as 413 accessions of *O. sativa* diversity panel, have been believed representing the genetic diversity of the primary gene pool of predominantly domesticated rice (Ali et al. 2011), has been genotyped with 44,000 SNPs (10 SNPs/kb) as a basis for GWAS (Zhao et al. 2011; Tung et al. 2010; McCouch et al. 2010). The slow decay of LD, while facilitating GWAS analysis, can limit the studied resolution of rice association mapping. The first targeted association mapping study in rice (Garris et al. 2003) showed that LD decay in a subpopulation covers about 90 kb (five genes) in a region of chromosome 5 containing the *xa5*. LD is thought to decay more rapidly in *O. rufipogon* (50 kb, or 1–3 genes) (Rakshit et al. 2007), provides higher resolution for LD mapping, and more slowly in rice japonica subpopulation (Mather et al. 2007; Rakshit et al. 2007; McNally et al. 2009). However, when compared to QTL study resolution (250 lines) (10–20 cM density, where 1 cM = 250 kb), association mapping is expected to provide between 10 and 200 times tighter resolution for populations of the same size over marker densities, which is sufficient to take advantage of the recombination event. Thus, a GWAS using marker densities similar to the QTL study would not have an increased resolution and will increase the risk of type-2 error. For GWAS and QTL analysis in rice, fine-mapping and/or mutant studies are generally required to identify the genes underlying the QTL of interest. However, fine-mapping of rice loci can generally focus on a smaller target area in the GWAS analysis.

Recently, a total of 48 distinct genomic regions of Al tolerance were detected by GWAS mapping based on root relative growth (Famoso et al. 2011). The region on chromosome 2 containing the candidate gene *Nrat1* (Os02g0131800), is the same as the QTL location for Al tolerance on chromosome 2 that was detected previously in a population from Kasalath and Koshihikari (Ma et al. 2002). This QTL describes the 7.3% variation in Al tolerance. However, the genes responsible for QTL have not been identified.

1.3.6 Al-Tolerance Regulating Genes in Rice

Al stress tolerance in Triticeae and Poaceae members is a qualitative character, indicating this trait was controlled by several genes (Table 1.4). Al stress tolerance in Triticeae members is a qualitative character. Some of the Al tolerant gene loci that have been detected by mapping techniques in Triticeae members are Alt1 or AltBH in wheat (Delhaize et al. 1993; Kochian 2000; Budzianowski and Wos 2004), Alp on barley (Tang et al. 2000), and Alt3 in the rye (Aniol and Gustafson 1984; Miftahudin et al. 2002). In rice and *Arabidopsis*, Al stress tolerance is a quantitative character. It is difficult to analyze quantitative traits, but the availability of nucleotide sequences from the genomes of rice and *Arabidopsis* and their annotations facilitates and accelerates the discovery of genes underlying Al stress

tolerance in both plants (Kochian et al. 2004). Al stress tolerance related to several morpho-physiological traits in rice is controlled by several genes (Fig. 1.2).

Genes that has a vital role in Al tolerance are the *STOP1*, *STOP2*, *ASR5*, *WRKY46*, *ART1*, *STAR1*, and *STAR2*. The sensitivity to proton rhizotoxicity 1 (*STOP1*) gene was isolated from *Arabidopsis* and acts as a transcription factor with the Cys2/His2 zinc-finger type and regulates the transporter genes associated with Al tolerance (Iuchi et al. 2007). However, the *STOP1* gene failed to induce the *AtALMT1* gene. The *STOP2* gene is an Al tolerance gene that is controlled by STOP1 and its expression level is much lower than that of STOP1 (Kobayashi et al. 2014). The *ASR5* gene is an Al tolerance gene and acts as a transcription factor (Arenhart et al. 2014). However, the *ASR5* gene also plays a role in abscisic acid control and fruit ripening. The *WRKY46* gene is a gene that acts as a transcription repressor of the *ALMT1* gene (Ding et al. 2013).

The *ART1* (*Aluminum rhizotoxicity 1*) gene belongs to the Al tolerance gene that controls many other Al tolerance genes in rice (Yamaji et al. 2009). ART1 that located in the nucleus of the root cell regulates more than 31 genes via binding the cis-acting element [GGN(T/g/a/C)V(C/A/g)S(C/G)] in the promoter of those genes (Tsutsui et al. 2011). *ART1* expression is not induced by Al^{3+}, but downstream gene expressions were up-regulated by Al within hours. Eight ART1-regulated genes, i.e., *STAR1*, *STAR2*, *OsMGT1*, *Nrat1*, *OsALS1*, *OsFRDL4*, *ART2* and *OsCDT3* were functionally characterized. *STAR1* (Sensitive to Al rhizotoxicity1) and *STAR2* (Sensitive to Al rhizotoxicity2) genes encode the ATP-binding domain and membrane-binding domain, respectively, components of the bacterial-type ATP-binding cassette (ABC) transporter that is expressed primarily in the roots (Huang et al. 2009a, b, c). The STAR1–STAR2 complex is localized to vesicles that transport UDP-glucose, which is involved in modification of cell wall, thereby decreasing Al accumulation in the cell compartment. Both *STAR1* and *STAR2* genes are transcriptionally activated by Al and silencing of those genes causes rice sensitivity to Al. *STAR1* and *STAR2* genes are similar to the two Al-sensitive mutant genes in *Arabidopsis*, *als1*, and *als3*, which also encode the ABC transporter (Larsen et al. 2005, 2007). OsMGT1 has a role as an Mg transporter (Chen et al. 2012a, b) and the up-regulation of *OsMGT1* causes internal Al toxicity is overcome by increasing Mg uptake. *Nramp aluminum transporter 1* (*Nrat1*) is one of the genes whose expression is controlled by an ART1 transcription factor and encodes an Al transporter localized to the root cell membrane (Huang et al. 2009a, b, c; Yamaji et al. 2009; Xia et al. 2010). The Nrat1 protein is thought to modulate Al tolerance via transporting Al into cells, reducing the Al concentration in the cell wall, and sequestrating or subsequent accumulating the Al^{3+} into the vacuole for final detoxification. The vacuole sequestration of Al^{3+} is mediated by *OsALS1*, a semi-sized ABC-transporter localized in rice tonoplast (Huang et al. 2012a, b).

OsFRDL4 is responsible for gene encoding citrate secretion, particularly, in response to Al^{3+} (Yokosho et al. 2011), while a cysteine-rich protein (OsCDT3) exhibits binding activity with Al^{3+}, thereby preventing Al^{3+} from entering root cells (Xia et al. 2013). Among those genes studied, *OsFRDL4* has a high correlation between its level of Al expression and tolerance (Yokosho et al. 2011), suggesting

Table 1.4 Genes responsible for aluminum tolerance in rice

Gene	Gene description	Phenotype alteration	References
ART1	Al resistance transcription factor 1	Encodes a C2H2 transcription factor and regulates other Al-tolerance genes in rice; classified as a constitutive gene; localized in the nucleus of all root cells; enhance Al tolerance in rice	Yamaji et al. (2009), Tsutsui et al. (2011), Ma et al. (2014)
ART2	Al resistance transcription factor 2	Encodes a transcription factor and controls multiple genes correlated to Al tolerance in rice; has motives C2H2 zinc finger; localized in the nucleus of all root cells; the expression level is affected by Al treatment (inducible); enhance Al tolerance in rice	Che et al. (2018)
ASR5	The abscisic acid, stress and ripening 5	Has roles as a key transcription factor and acts as Al tolerance gene in rice; interacts to the *STAR1* promoter and other Al-responsive genes	Arenhart et al. (2014), Ma et al. (2014)
ASR1	The abscisic acid, stress and ripening 1	Has dual roles both in the cytoplasm and nucleus, particularly acts as chaperones and as transcription factors; acts (together with ASR5) in concert and complementarily to control gene expression to Al-stress	Arenhart et al. (2016)
STAR1	Sensitive to Al rhizotoxicity 1	Has a nucleotide-binding domain of a bacterial-type ATP binding cassette (ABC) transporter; participates in detoxification of Al; has the mode of action as transporter and rice cell wall modification via transports UDP-glucose	Huang et al. (2009a, b, c), Ma et al. (2014)
STAR2	Sensitive to Al rhizotoxicity 2	A trans membrane domain of a bacterial-type ATP binding cassette (ABC) transporter; detoxifies Al in the cell wall like *STAR1*	Huang et al. (2009a, b, c), Ma et al. (2014)
FRDL4	Ferric reductase	Encodes a citrate efflux transporter; controls citrate	Yokosho et al. (2016), Ma et al. (2014)

(continued)

Table 1.4 (continued)

Gene	Gene description	Phenotype alteration	References
	defective 3-like 4	secretion in rice roots and chelate Al to be Al-citrate complex; has ART1 cis-acting elements in the 1.2-kb of *OsFRDL4* promoter and continuously increases the expression level; acts as an inducible gene in rice ssp. Japonica	
ALS1	Aluminum sensitive 1	Al transporter localized at the tonoplast; detoxify Al-toxicity	Ma et al. (2014)
ALS3	Aluminum sensitive 3	Al transporter localized at the tonoplast; detoxify Al-toxicity	Ma et al. (2014)
Nrat1	N ramp aluminum transporter 1	Involves as a transporter specific for Al; the expression is up-regulated by Al; highly expressed in the roots; controlled by an ART1 transcription factor; has a mode of action in Al detoxification via sequestration of Al into vacuoles	Xia et al. (2010), Ma et al. (2014)
WRKY22	WRKY family transcription factor	Increases Al tolerance through activation of *FRDL4* expression; enhances the citrate secretion in rice root; acts as a regulator in Al tolerance	Li et al. (2018a, b)
GERLP or B11	Gene encoding ribosomal L32-like protein	Increased Al-tolerance in rice and isolated from tropical Japonica subspecies rice especially cv. HawaraBunar; acts as a regulator for other Al-responsive genes	, Miftahudin et al. (2021), Siska et al. (2017), Ratnasari et al. (2016), Fendiyanto et al. (2019a), Fendiyanto et al. (2019b)

that this gene contribute to the genotype differences in Al toxicity tolerance. Conversely, there was no correlation among variations in genotype particularly differential gene expression of *STAR1*, *OsMGT1*, *OsCDT3*, and *OsALS1* to Al tolerance (Huang et al. 2009a, b, c, 2012a, b; Chen et al. 2012a, b; Xia et al. 2013), concluding that the genes are involved in the general fundamental detoxification process of Al in broad rice varieties.

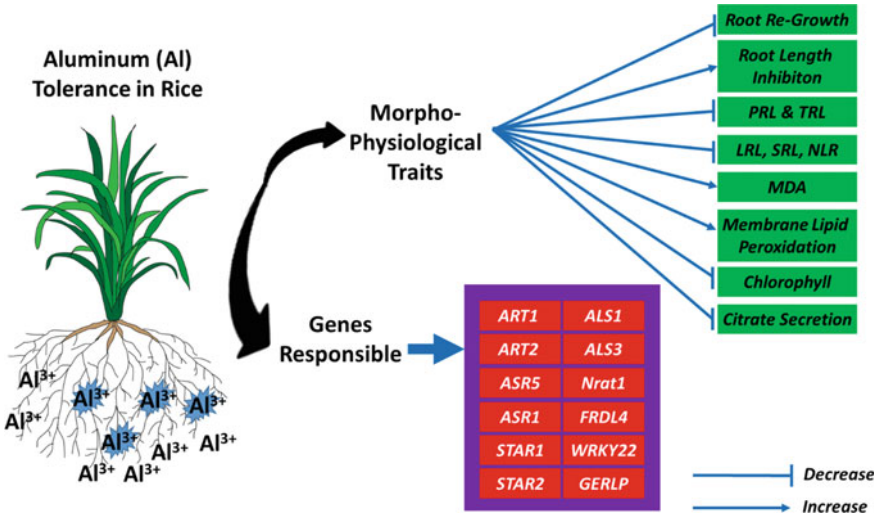

Fig. 1.2 Morpho-physiological and genes are responsible for aluminum (Al) tolerance in rice. PRL: primary root length; TRL: total root length; LRL: lateral root length; SRL: seminal root length; NLR: number of lateral roots; MDA: malondialdehyde

Another Al related tolerance gene, called *OsGERLP*, has been successfully isolated from rice cv HawaraBunar, a local cultivar in Indonesia. The gene was cloned based on the microsynteny relationship between rye chromosome 4RL and a rice BAC clone from chromosome 3. Miftahudin et al. (2004, 2005) have developed several molecular markers based on nucleotide sequence information from rice genomic DNA to make high-resolution mappings of the *Alt3* gene region in the rye. Among the markers, there are two markers, i.e., B6 and bcd1230, flanked the Alt3 locus very tightly and spanning 50 kb region in the rice BAC clone. In silico analysis reveals that there is four potential candidate gene in between both markers, and one of them called *B11* gene showed a potential Al tolerance gene (Roslim 2011). Gene expression analysis showed that the gene expression is upregulated by Al and express higher in Al-tolerant rice than that in the Al-sensitive rice.

The *B11* gene encodes a ribosomal bacterial L32-like protein that also contains a C2H2-like motif which could be a transcription factor (Roslim 2011). The gene was then named *OsGERLP*. Silencing the gene in Al-tolerant rice could impair root growth under Al stress conditions, conversely, overexpression of the gene in tobacco could increase Al tolerance of the transgenic tobacco (Miftahudin et al. 2021). In addition, a SNP marker derived from the gene was able to distinguish the level of Al tolerance among rice lines in the IR64 × HawaraBunar derived RIL population as well as cultivars of Inpago rice (Fendiyanto et al. 2019a, b). Based on the characteristic of the gene, it is suggested that the *OsGERLP* could be included in the Aluminum tolerance gene that might be contributed to Al tolerance in rice.

1.3.7 Breeding Studies of Al Tolerance in Rice

1.3.7.1 Understanding RILs in QTL Mapping

Recombinant inbred lines (RILs) are a set of lines that are homozygously produced by continuous self-pollination (selfing or inbreeding) of F2 crossovers (Burr et al. 1988; Simpson 1989; Burr and Burr 1991). RIL population is also known as F2-derived inbred lines. In addition, the RIL population can also be referred to as single seed descent (SSD) because it is derived from the F2 population by the SSD procedure. The first discovery of the concept of using RILs in linkage mapping was carried out on mice with 20 generations from sibling results which resulted in different levels of homozygosity (Schneider 2005). The homozygosity level in RIL resulted in sib-mating two times higher than that of self-pollinated crosses. This can occur because the chance of heterozygosity in self-pollination (selfing) is 50%, while the chance of genetic constitution in a sibling is reduced to 25%. Thus, selfing requires half the sibling to achieve the same homozygosity. Additionally, sib-mating would require twice as many F2 plants as selfing to produce the same amount of RIL. SSD protocols are the most suitable way to develop RIL, but the bulk and pedigree method, particularly natural population without selection can also be performed. What is necessary for making RIL is that the rate of generation progression must be performed under an optimal soil that provides the same survival and good adaptation of multiple varieties and does not create precisely selection pressure for various varieties.

RIL has been used in many crops, and some population of RILs has become a mapping tool to find QTLs, for example, more than 300 RILs population generated from crosses between *Landsberg erecta* and *A. thaliana* (Columbia ecotypes) (Lister and Dean 1993). RIL has been widely developed for the generating of genetic maps according to molecular markers; identification of several markers associated with genes regulating qualitative traits such as vertical disease resistance, Al-tolerance, drought stress tolerance, Fe-toxicity tolerance, biotic or abiotic stresses, seed color, flower color, flower type, seed shape, and fruit shape. Detection of markers associated with QTL that are responsible in controlling many traits such as abiotic stress resistance, crop yields, flowering time, QTL mapping, and incorporation of gene or QTL maps with molecular marker maps. The use of RIL populations has recently been developed epigenetically. Many researchers have succeeded in developing epigenetic recombinant inbred populations (epiRIL). The Arabidopsis epiRIL population was identified by crossing two distinctly parental lines showing slight differences at the level of DNA sequences, but contrasting patterns in methylation of DNA (Johannes et al. 2009).

1.3.7.2 Differences in Al Tolerance Levels Among Rice Varieties

There are genotypic variations in Al tolerance in rice both temperate japonica, indica, aus, and tropical japonica. Typically, japonica (tropical or temperate) cultivars show a higher Al tolerance than indica cultivars, which are cultivated in acid soils. More than 10 QTLs for Al tolerance have been identified (Wu et al. 2000; Nguyen et al. 2001, 2002, 2003; Ma et al. 2002; Xue et al. 2007; Fendiyanto et al. 2019b), but the gene for the QTL hasnot been cloned so far except *OsGERLP* and *ART1*. Among the genes controlledby ART1, such as Os01g0869200, Os01g0919200, Os02g0131800, Os07g0587300, Os11g0488100, and Os11g0490100, are in the same position as the QTL region for Al tolerance properties. All of them can be found on chromosomes 1, 2, 7, and 11 (Ma et al. 2014). Ma et al. (2014) suggesting that these genes may be responsive in genotypic differences in Al tolerance level in broadly rice varieties, however, it needs further confirmation. Siska et al. (2017), Fendiyanto et al. (2019a, b) identified that the HawaraBunar variety, which is included in the tropical japonica subspecies, is an Al-tolerant, while IR64 is an Al-sensitive variety. In addition to genotypic differences, some upland rice such as Inpago 4-11, Jatiluhur, Situ Patenggang, and Situ Bagendit are classified as sensitive to moderate varieties based on the study of *SnpB11* markers, morphophysiology, and MDA in the germination phase (Fendiyanto et al. 2019a).

1.3.8 Latest Advanced Molecular Genomic Techniques in the Comprehensive Study of Aluminum Tolerance in the Present and Future

Approaches to characterize genes or loci for Al tolerance properties can be performed in several ways, i.e., by making transgenic plants, in particular by genetic engineering and introgression of aluminum-related genes into Al-sensitive rice varieties. The gene characterization process by making transgenic plants is carried out by inserting certain genes into Al-sensitive rice or the specific mutant rice. This technique has been used by Yamaji et al. (2009) when they characterize the *ART1*. A similar technique was also adopted by Ratnasari et al. (2016) in introduced the *OsGERLP* from rice into tobacco as a model plant. The genetic engineering technique was also performed by Wahyuningtyas et al. (2016) in making Al-silenced transgenic rice of *B11*, a candidate gene for Al tolerance in HawaraBunar variety using RNA interference technique. In addition to plant genetic engineering technology, loci introgression techniques to rice varieties that are difficult to transform can also be used as an alternative to characterize genes related to Al tolerance. Wijayanto (2013) conducted an introgression of Al tolerance genes with a marker-assisted backcrossing technique using the BC2 backcrossing population. In addition to techniques in gene characterization, the newest technique that can be

performed in the future is by utilizing genome editing through the CRISPR/Cas-9 technique and integrated omic-technologies such as genomics, transcriptomics, and metabolomics. The two new techniques can help to comprehensively discover and understand Al-related genes including their complex mechanism in rice.

1.4 Genetic Improvement of Rice for Fe Toxicity Tolerance

1.4.1 Iron, Soil, and Plants

Iron (Fe) is the fourth most abundant element contained in the earth's crust and important for living things. Various important processes in plants involve Fe. Iron is one of the constituent elements of various enzymes and proteins. Several flavoproteins form bonds with Fe^{3+}, e.g., succinic $FADH_2$ dehydrogenase, dihydroorotic acid dehydrogenase, xanthine, and aldehyde oxidase. Fe also acts as a structural component of several molecules with porphyrin rings, such as cytochromes, heme, ferrichrome, and leghemoglobin. In addition, most of the enzymes containing Fe are involved in redox reactions in the respiration and photosynthesis processes (Briat and Lobréaux 1997; Brumbarova and Bauer 2008; Mitra 2015). Iron is also involved in the biosynthesis process of chlorophyll and coenzymes containing heme (Marschner 1995).

In the soil, Fe presents as the ferrous ion (Fe^{2+}) and ferric ion (Fe^{3+}) and Fe availability differ between alkaline and acidic soil conditions. The Fe availability in the soil depends on the redox potential (Eh), soil acidity level (pH), amount of organic matter, and the waterlogging duration. Fe as ferrous ion up to more than 1,000 ppm presents in acid soils with high organic matter, whereas in alkaline soil types, the concentration could be less than 20 ppm (Onaga et al. 2016). Iron status either in acidic or alkaline soil can cause a nutritional imbalance in plants. Iron deficiency causes chlorosis in plants, while iron excess triggers oxidative stress which can cause permanent damage at the cellular and tissue level (Jeong and Connolly 2009; Zheng 2010).

The critical value of Fe that creates toxic conditions for rice is 300 ppm in leaf blades at the tillering phase (Yoshida et al. 1976). However, the amount of Fe required known varies depends on plant variety and soil type. Becker and Asch (2005) stated that Fe concentration ranges from 10 to more than 2,000 ppm in soil solutions and 20–2,500 ppm in plants cause Fe toxicity symptoms. The high concentration of Fe^{2+} in the soil solution has an impact on the inhibition of other nutrient absorption, such as P and K (Yoshida 1981). Hence, to reach optimal plant growth, Fe concentration ranges from 10^{-9} to 10^{-4} M are required (Guerinot and Ying 1994). When iron concentration in solutions more than 200 ppm, it leads to toxicity stress to rice (Noor et al. 2012; Elec et al. 2013; Nugraha et al. 2016; Turhadi et al. 2018).

Permanent damage at cellular and tissue levels and leads to cell death in plants due to oxidative stress can also be triggered by Fe excess. Marschner (1995) states that in an aerobic conditions, the Fe presents abundant and will produce free radicals or reactive oxygen species (ROS) in the form of oxygen $(O_2^{\cdot -})$ and hydroxyl radicals $(OH^{\cdot})$. These free radical compounds induce lipid peroxidation that causes cell membrane damage.

1.4.2 Iron Transport and Metabolism

In plants, the mechanism of Fe uptake and transport has been well characterized. The Fe transport in plants is known to involve various processes that are controlled by transporter proteins and also certain genes. Finatto et al. (2015) classify the genes controlling Fe transport into three groups based on their putative function in regard to maintain Fe homeostasis, i.e., Fe uptake and mobilization group, Fe transport group, and regulatory mechanism group. These genes are regulated in the process of Fe uptake from the rhizosphere, distribution among tissues and plant organs, and Fe storage in the organelles.

Krohling et al. (2016) reviewed two Fe uptake strategies and special uptake mechanisms in rice as categorized as grass plants. Generally, there are two strategies, known as strategy I and strategy II, developed by plants to absorb Fe from the rhizosphere (Kobayashi and Nishizawa 2012). The strategy I is found in the dicot and monocot plants, except the grass family, whereas strategy II is only found in grasses (Jeong and Connolly 2009). Both strategies involve the transport process from Fe absorption in the rhizosphere to the shoot through vascular tissues (Conte and Walker 2011; Bashir et al. 2013). However, rice adapts a strategy I-like mechanism to absorb Fe from the rhizosphere.

The Fe absorption through strategy I is mainly characterized as a reduction strategy. Acidification by H^+-ATPase (P-ATPase) in the plasma membrane increases the Fe^{3+} solubility level in the rhizosphere. Then, a reduction mechanism catalyzed by ferric chelate reductase (FRO) enzyme occurs to convert Fe^{3+} to Fe^{2+}. The Fe^{2+} will enter root cells through iron-regulated transporter 1 (IRT1) in the plasma membrane of root epidermal cells (Kobayashi and Nishizawa 2012; Krohling et al. 2016).

Strategy II for Fe uptake in plants has been well characterized as a chelation strategy using the root-released compound as a chelator. There are several important chelators that capable to chelate Fe, namely nicotinamide (NA), mugenic acid (phytosiderophore), citric acid, and phenolic compounds. Among those chelators, only mugenic acid is produced by grasses, while the other chelators are produced by non-grass plants (Bashir et al. 2013). As phytosiderophore (PS), mugenic acid could interact with Fe^{3+} in the soil. There are some representative mugenic acid family members, includes 2′-desoximugineic acid (DMA), 3-3-epihidroximugineic acid (epiHMA), and 3-epihydroxy-2′-deoxymugineic acid (epiHDMA) (Krohling

et al. 2016). Rice uses mugenic acid as a chelator to absorb Fe (Mitra 2015). In strategy II, TOM1 transporters mediate PS secretion to the rhizosphere area through the root plasma membrane. The complex PS-Fe(III) formed in the rhizosphere is then entered into root cells via YS1 transporter.

Both reduction (a strategy I) and chelation (strategy II) are involved in rice to absorb Fe from the rhizosphere, which is characterized as a combination strategy. This combination strategy is probably associated with rice adaptation in two conditions, flooded (anaerobic) and aerobic (Ricachenevsky and Sperotto 2014). When using the strategy I, rice absorb Fe^{2+} via Fe^{2+} transporters (*OsIRT1* and *OsIRT2*) (Ishimaru et al. 2006), while in strategy II, rice secretes phytosiderophore compound (deoxymugineic acid/DMA) as a chelator to bind Fe^{3+} that mediated by efflux transporter, TOM1 (Nozoye et al. 2011). The Fe^{3+}-DMA complexes are then absorbed to the root cells through YS1-like protein in the plasma membrane, OsYSL15 (Inoue et al. 2009).

1.4.3 Iron Toxicity Stress and Its Effect on Rice

Iron is a micronutrient that is required by plants in a small amount. When Fe available and excessively absorbed by the plant, it causes a negative or toxic effect. Iron toxicity stress in plants is characterized by visual bronzing or dark necrotic spots in leaf blades. The bronzing develops starting from the tip of the oldest leaf blade and then spreads to all parts of the leaf in line with the stress duration of Fe toxicity stress (Aung et al. 2018). Fe toxicity could be one of the most important abiotic stress in rice cultivation with poorly drained lowland or submerged areas and low soil pH that allows direct contact of the root system to the wetland soil (Dobermann and Fairhurst 2000). During the flooding period of rice cultivation, limited diffusion of atmospheric oxygen into the soil promotes a hypoxic condition and induces a reduction state in the soil (Krohling et al. 2016).

The Fe toxicity stress in rice can occur at various growth stages, from seedling, vegetative, and reproductive stages. The leaf bronzing as a result of Fe toxicity stress is associated with growth reduction and yield failure. In anaerobic and low pH conditions of submerged soils, Fe^{3+} is converted to a more soluble form, Fe^{2+}. Excessive Fe^{2+} uptake by roots will be transported to the shoots through xylem vessels and causes oxidative stress due to ROS overproduction from Fenton and Haber-Weiss reactions. This oxidative stress causes physiological disorders (cellular structure, DNA, and protein damages). Fe toxicity then causes a bronzing symptom in leaves, which is in serious conditions it will be followed by complete yield failure (Aung and Masuda 2020).

As a serious nutritional problem in agriculture, Fe toxicity stress seriously inhibits plant growth, reduces photosynthesis activity, increases lipid peroxidation, changes metabolite profiles, and decreases yield (Chatterjee et al. 2006; Elec et al. 2013; Turhadi et al. 2019, 2020). Nutrient absorption in plants under Fe toxicity stress is inhibited, hence it leads to deficiencies in several essential nutrients (P, K,

Mg, Ca, Mn, and Zn) (Onaga et al. 2016). For example, Mn concentration in potato variably and significantly decreased with the increasing Fe supply ranged from 0.001 to 2.0 mM (Chatterjee et al. 2006).

1.4.4 Tolerance Mechanism to Iron Toxicity Stress

Environmental conditions with Fe excess have caused plants to develop several tolerance strategies. A comprehensive model of tolerance strategies to Fe toxicity stress in rice has been reported by Aung and Masuda (2020). Rice responds to Fe toxicity stress comprised of four defense strategies, which are called defense 1, 2, 3, and 4. **Defense 1**, rice plant develops Fe exclusion strategy from the roots. **Defense 2**, rice plant retains Fe in the roots and suppresses Fe translocation to the shoots when exposed to Fe toxicity stress condition. **Defense 3**, rice plant compartmentalize Fe in the shoot as a mitigation strategy under Fe toxicity stress condition. **Defense 4**, rice plant develops a ROS detoxification strategy to suppress the oxidative damage effects as a consequence of Fe toxicity stress condition. Notwithstanding that four tolerance strategies to Fe toxicity stress as proposed by Aung and Masuda (2020), in general, there are three types of tolerance strategies for Fe toxicity stress in plants, namely excluder-avoidance, includer-avoidance, and includer-tolerance (Becker and Asch 2005).

The excluder-avoidance type is characterized by a strategy to prevent Fe^{2+} ions from excessively enter the roots by increasing the ability of root oxidation in the rhizosphere (defense 1) and Fe retention in the roots (defense 2). Aung et al. (2018) reported that genes for Fe-uptake, Fe-transport in the roots, and biosynthesis of mugenic acids, such as *OsIRT1*, *OsIRT2*, *OsYSL2*, *OsYSL15*, *OsNRAMP1*, *OsNAS1*, *OsNAS2*, *OsNAAT1*, and *OsDMAS1*, were suppressed under Fe toxicity stress. In addition, Hemerythrine motif-containing Interesting New Genes (*OsHRZ1* and *OsHRZ2*) were also reported as important genes for maintaining Fe homeostasis in plants under Fe toxicity stress through the repression mechanism of genes involved in Fe uptake and translocation. Discrimination Center (DC) as part of the basal area of the shoot is suggested to have an important role in tolerance strategies of rice under Fe toxicity condition. Fe transport-related genes such as *OsIRT1*, *OsYSL2*, *OsTOM1*, *OsNRAMP1*, and *OsYSL15* are highly suppressed in DC. This condition suggests that those genes are likely retaining Fe in the roots and the DC, and then distribute less Fe to the shoots (Aung and Masuda 2020).

The includer-avoidance type is characterized by the involvement of a compartmentation strategy in the organelles, such as vacuoles and chloroplasts (defense 3), hence the absorbed Fe does not cause excessive toxic effects. Under excessive Fe, certain genes such as *OsNAS3*, *OsVIT2*, and rice *ferritin* genes (*OsFers*) are highly expressed in various tissues (Aung et al. 2018). Those genes may be important for compartmentation strategy when plants under Fe toxicity.

The includer-tolerance type is characterized by the involvement of the ROS detoxification strategy using defense 4. The strategy of Fe^{2+} exclusion on the root

surface, Fe compartmentation in shoots, and ROS detoxification are assumed as main mechanisms that are involved in plant tolerance to Fe toxicity stress (Becker and Asch 2005; Engel et al. 2012; Wu et al. 2016). Antioxidative enzymes involved in ROS detoxification have an important role to protect plants from oxidative stress that arises from Fe toxicity stress (Devi et al. 2016). Moreover, excess absorption of Fe does not cause oxidative stress due to ROS, Fe will bind with other elements to form a heme and non-heme protein complex (Marcshner 1995). Various oxygen and electron transfer related-genes, cytochrome P450 family proteins, or some NAC-type transcription factors (*OsNAC4*, *OsNAC5*, and *OsNAC6*) were induced under Fe excess conditions to avoid excess ROS (Aung et al. 2018). A transcription factor *WRKY* is also suggested to be related to plant response to Fe toxicity stress. Ricachenevsky et al. (2010) showed that the expression level of *OsWRKY80* increases under Fe excess conditions. Increased expression of *OsWRKY80* occurred in all vegetative organs of rice (roots, stems, and leaves). However, further comprehensive studies are still required to investigate the specific roles of *WRKY* in Fe toxicity tolerance. An*S-nitrosoglutathione-reductase* (GSNOR) is also reported as an important part of the tolerance strategy to Fe toxicity stress, especially in roots (Li et al. 2019).

Defense system to scavenge the produced ROS by plants under stress conditions, various enzymes were also involved. To avoid excess oxidative damage under Fe toxicity stress conditions, *Euglena uniflorous* developed a well-organized antioxidative defense system using superoxide dismutase (SOD), peroxide dismutase (POX), glutathione reductase (GR), guaiacol peroxidase (GPX), catalase (CAT) enzymes, and also ascorbic acid and reduced glutathione reductase (GSH) (de Oliveira Jucoski et al. 2013).

1.4.5 Recent Advances of Breeding Strategy for Improving Rice Tolerance to Fe Toxicity Stress

1.4.5.1 Screening Methods for Rice Phenotyping Under Fe Toxicity Stress

There are four screening methods for rice phenotyping to obtain tolerant genotypes to Fe toxicity (Sikirouet al. 2015). Laboratory (controlled growth chamber), greenhouse, and field experimental studies have been chosen for the screening strategies. Field screening under Fe toxicity (Method 1), pot screening using Fe-toxic soils (Method 2), pot screening using washed sand soil that supplemented with exogenous Fe sources (Method 3), and hydroponic culture nutrient solution supplemented with exogenous Fe sources (Method 4) is the screening methods for Fe toxicity tolerance in rice. Each screening method has advantages and disadvantages (Table 1.5). Methods 1, 2, and 3 could be used for screening at any

growth stage of rice plants until maturity, whereas method 4 only suitable for screening at the early vegetative growth stage.

Sites with Fe toxicity are the areas with a high level of Fe content that can seriously affect rice production. Screening method 1 should be conducted in natural Fe-toxic fields. Becker and Asch (2005) classified the Fe-toxic site into three clusters. Coastal planes and river deltas area types with young acid sulfate soils such as in Vietnam, Liberia, Senegal, and Thailand are categorized as cluster I of Fe-toxic field with 500–2500 ppm of Fe. Marches and highland swamps area types with clays Ulti- and histosols such as in the Philippines, Indonesia, Burundi, and Madagascar are categorized as cluster II of the Fe-toxic field with 300–900 ppm of Fe. Inland valley swamps area types with sandy valley-bottom soils such as in Guinea, Madagascar, Cote d'Ivoire, and Sri Lanka categorized as cluster III of Fe-toxic field with 20–600 ppm of Fe. These three clusters could be used as reference sites for field screening under Fe toxicity conditions. Stein et al. (2014) reported that soil Fe concentration in the Camaqua, Southern Brazil was 284 mg/L and success to investigate different mechanisms of two tolerant rice cultivars to Fe toxicity under field conditions. Field evaluation of several Indonesian rice genotypes to Fe toxicity was also conducted in the Fe-toxic soil with 177–200 ppm in Lampung, Indonesia (Utami and Hanarida 2014). However, soil heterogeneity, the interaction between genotype and environment (G × E), labor techniques, and season were major problems for this method. As alternative methods, the growth chamber and greenhouse could be the choices for the screening methods without depends on the local season.

1.4.5.2 QTLs Basis of Fe Toxicity Tolerance

The development of tolerant rice varieties to Fe toxicity is still in progress. In rice, the Fe toxicity tolerance trait is controlled by quantitative trait loci (QTLs) distributed in all rice chromosomes. Various traits of Fe toxicity stress tolerance in rice have been reported by several research teams worldwide. There were 37 Fe tolerance related traits identified based on QTLs located in 12 rice chromosomes (Table 1.6).

Among the various traits identified, leaf bronzing index or also known as leaf bronzing score, and plant height are the two identified QTL traits located on all rice chromosomes. Furthermore, among 12 rice chromosomes, chromosomes 1, 2, 3, and 7 showed a high number of identified traits for Fe toxicity stress tolerance in rice, with the amount of 27, 21, 22, and 19 traits per chromosome, respectively. Based on these findings, acceleration of the breeding program for tolerant rice varieties to Fe toxicity stress could focus on those traits and chromosome regions. Moreover, nowadays QTL analysis of Fe toxicity tolerance traits in rice has covered almost all important traits in breeding strategies.

Table 1.5 Various screening methods for Fe toxicity stress

	Field screening under Fe toxicity (1)	Pot screening using Fe-toxic soils (2)	Pot screening using washed sand soil supplemented with $FeSO_4.7H_2O$ (3)	Hydroponic culture nutrient solution supplemented with $FeSO_4.7H_2O$ or Fe-EDTA or $FeCl_3$ (4)
Data collection time	Any growth stage till maturity			Early vegetative growth stage
Measurable trait	• Plant height • Shoot biomass • Yield • Bronzing score • Tissue Fe concentration • Gas exchange parameters • Basic agronomic traits	• Plant height • Shoot biomass • Yield • Bronzing score • Tissue Fe concentration • Gas exchange parameters	• Plant height • Shoot biomass • Yield • Bronzing score • Tissue Fe concentration • Gas exchange parameters	• Plant height • Root and shoot biomass • Root characteristics • Bronzing score • Tissue Fe concentration • Gas exchange parameters
Advantage	Direct measurement of yield	• Direct measurement of yield • Screening can be done in both wet and dry season	• Direct measurement of yield • Screening can be done in both wet and dry season	• Easiness • A short period (2–8 weeks) • High throughput
Disadvantage	• Soil heterogeneity • G × E interaction • Labor and space • In rainfed condition, the trial cannot be done in the dry season	• Labor and space • Transportation of soils	• Labor for washing soils • Space • Micronutrient requirement	• No measurement of yield
References	Sahrawat and Singh (1998), Audebert (2006), Camara (2006), Gridley et al. (2006), Nozoe et al. (2008), Utami and Hanarida (2014), Stein et al. (2014), Turhadi et al. (2020)	Abifarin (1989), Yamauchi (1989)	Dufey et al. (2012), Onaga et al. (2013)	Audebert and Sahrawat (2000), Shimizu et al. (2005), Elec et al. (2013), Ruengphayak et al. (2015), Dufey et al. (2015b), Matthus et al. (2015), Nugraha et al. (2016), Zhang et al. (2017), Turhadi et al. (2018)

Adapted from Sikirou et al. (2015) with minor modification

Table 1.6 Quantitative trait loci identified for Fe toxicity stress tolerance in rice

No	Traits	Chromosome	Population	References
1	Ascorbate peroxidase activity	1	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
2	Ascorbate content	1	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
3	Chlorophyll content index	7	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		1, 2, 3, 4, 7, 11	IR64 × Azucena	Dufey et al. (2009)
		2	Caiapo × MG12	Dufey et al. (2015b)
		3	Caiapo × MG12	Dufey et al. (2015b)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
4	Chlorophyll content	3	Kinmaze × DV85	Wan et al. (2004)
		1, 2, 3, 4, 5, 6, 8, 9, 11, 12	Longza 8503 × IR64	Wan et al. (2005)
5	Chlorophyll fluorescence	2, 7	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
6	Coleoptile elongation rate	1, 4, 5, 7	Zhenshan 97B × Miyang 46	Ouyang et al. (2007)
7	Dehydroascorbate activity	1	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
8	Fertility rate	3, 7	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
9	Growth cycle length	3	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
10	Glutathione reductase activity	1	Integrative map of 14 studies using different populations	Dufey et al. (2015a)

(continued)

Table 1.6 (continued)

No	Traits	Chromosome	Population	References
11	Leaf bronzing index	1, 2, 3	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		1	IR29 × Pokkali	Wu et al. (2014)
		4	IR29 × Pokkali	Wu et al. (2014)
		1, 3, 8	Nipponbare × Kassalath	Wu et al. (2014)
		1, 8	IR64 × Azucena	Wu et al. (1998)
		1, 2, 7	IR64 × Azucena	Wu et al. (1998)
		3, 6, 7, 9, 11, 12	IR24 × Asominori	Wan et al. (2003a)
		1, 3	Nipponbare × Kassalath	Wan et al. (2003b)
		3	Kinmaze × DV85	Wan et al. (2004)
		1, 2, 3, 4, 5, 6, 8, 9, 11, 12	Longza 8503 × IR64	Wan et al. (2005)
		3, 4, 5, 8, 10	Bao Thai × Suakoko 8	Elec et al. (2013)
		1, 2, 7, 8	Gimbozu × Kassalath	Shimizu (2009)
		1, 2, 3, 4, 7, 11	IR64 × Azucena	Dufey et al. (2009)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
		7	IR29 × Pokkali	Wu et al. (2014)
12	Leaf blade Fe concentration	3	Caiapo × MG12	Dufey et al. (2015b)
13	Leaf Fe concentration	3	Integrative map of 14 studies using different populations	Dufey et al. (2015a)

(continued)

Table 1.6 (continued)

No	Traits	Chromosome	Population	References
14	Leaf-sheath Fe concentration	3	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		3	Caiapo × MG12	Dufey et al. (2015b)
		3, 4, 5, 8, 10	Bao Thai × Suakoko 8	Elec et al. (2013)
15	The logarithmic function of leaf bronzing index	5	Caiapo × MG12	Dufey et al. (2015b)
16	Non-photochemical quenching	1	Caiapo × MG12	Dufey et al. (2015b)
17	Number of spikelets per panicle	3	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
18	Panicle dry weight	3	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
19	Plant height	3	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		3, 6, 7, 9, 11, 12	IR24 × Asominori	Wan et al. (2003a)
		3	Kinmaze × DV85	Wan et al. (2004)
		1, 2, 3, 4, 5, 6, 8, 9, 11, 12	Longza 8503 × IR64	Wan et al. (2005)
		3, 4, 5, 8, 10	Bao Thai × Suakoko 8	Elec et al. (2013)
20	Root dry weight	1, 3, 7	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		3, 6, 7, 9, 11, 12	IR24 × Asominori	Wan et al. (2003a)
		1, 3, 8	Nipponbare × Kassalath	Wu et al. (2014)

(continued)

Table 1.6 (continued)

No	Traits	Chromosome	Population	References
		1, 2, 3, 4, 7, 11	IR64 × Azucena	Dufey et al. (2009)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
		1, 2, 3, 4, 7, 11, 12	Koshihikari × Kassalath	Fukuda et al. (2012)
21	Root-plaque Fe concentration	1, 5	Caiapo × MG12	Dufey et al. (2015b)
22	Root length	7	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		3, 6, 7, 9, 11, 12	IR24 × Asominori	Wan et al. (2003a)
		1, 2, 3, 4, 5, 6, 8, 9, 11, 12	Longza 8503 × IR64	Wan et al. (2005)
23	Relative variation of root dry weight	1	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
24	Stem dry weight	3, 6, 7, 9, 11, 12	IR24 × Asominori	Wan et al. (2003a)
		1, 3, 8	Nipponbare × Kassalath	Wu et al. (2014)
25	Stomatal conductance	1	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		10	Caiapo × MG12	Dufey et al. (2015b)
26	Shoot dry weight	3	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		1, 8	IR64 × Azucena	Wu et al. (1998)
		3	Kinmaze × DV85	Wan et al. (2004)
		3, 4, 5, 8, 10	Bao Thai Suakoko 8	Elec et al. (2013)
		1, 2, 3, 4, 7, 11	IR64 × Azucena	Dufey et al. (2009)

(continued)

Table 1.6 (continued)

No	Traits	Chromosome	Population	References
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
		1, 2, 3, 4, 7, 11, 12	Koshihikari × Kassalath	Fukuda et al. (2012)
27	Shoot iron concentration	3	Koshihikari × Kassalath	Fukuda et al. (2012)
28	Shoot Fe concentration	2	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
29	Spikelet per panicle	1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
30	Stomatal resistance	1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
31	Shoot K concentration	2	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
32	Shoot P concentration	7	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
33	Shoot water content	1, 2, 3, 4, 7, 11	IR64 × Azucena	Dufey et al. (2009)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
34	Tiller number	1, 3, 8	Nipponbare × Kassalath	Wu et al. (2014)
35	Tissue Fe concentration	1, 2, 7	IR64 × Azucena	Wu et al. (1998)
36	Total plot biomass	7	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)
37	100-grain weight	1, 2	Integrative map of 14 studies using different populations	Dufey et al. (2015a)
		1, 2, 3, 7	IR64 × Azucena	Dufey et al. (2012)

1.4.5.3 Development Progress of Tolerant Rice Varieties to Fe Toxicity Stress

Fe toxicity is one of the rice cultivation problems and the breeding program to develop tolerant rice varieties is continuously in progress. The tolerance phenotype to Fe toxicity is strongly influenced by an interaction between the genotype × environment (G × E). Large genotype × environment and genotype × year (or season) interaction could result in slow progress in the development of new varieties (Onaga et al. 2016). Interaction between G × E suggests that the Fe toxicity tolerance in most rice varieties is site-specific. However, the development of Fe toxicity stress-tolerant varieties in rice plants is still an important goal. As we know, rice is one of the staple food in the major population in the world, especially in Asia. Mahender et al. (2019) stated that there were several desirable traits in developing Fe toxicity tolerant rice varieties, such as higher tiller capacity, medium plant height, early maturity, heavy panicle structure and shape, and higher grain yield. Table 1.7 showed a list of rice cultivar/varieties that tolerant to Fe toxicity stress with category moderately tolerant to tolerant level. Asia and Africa are two of the major continents with intensive breeding progress for tolerant rice to Fe toxicity stress. This fact may be related to site characteristics with iron-toxic environments as clustered by Becker and Asch (2005), such as Vietnam, Thailand, Philippines, Indonesia, Liberia, Senegal, Burundi, Madagascar, Guinea, Côte d'Ivoire, and Sri Lanka. The tolerant varieties are listed in Table 1.7 could be a donor or parental to develop new tolerant rice varieties with desirable agronomic traits.

1.5 Genomic Manipulation to Improve Rice Tolerance to Abiotic Stress

Agriculture and breeding have now started to enter the 4.0 era. Wallace et al. (2018) proposed that breeding 4.0 was a designed genome. Functional variants detected on QTLs and genes responsible for abiotic stresses identified by structural or functional genomics could be used to edit the rice genome to produce the phenotype of interest, including multi-tolerant abiotic stress traits. Especially in drought, Al, and Fe toxicity tolerance, QTLs regulate many variations in plant phenology and traits, including morpho-physiological mechanism. There were many physiological, genetic, and molecular studies to understand both globally and specific mechanisms for drought, Al, and Fe toxicity tolerance. However, the correlation between molecular mechanisms with yield is not fully understood. Novel gene discovery still needs to be done to identify the preciseness genes or QTLs associated with drought, Al, and Fe toxicity tolerance. Modern genomics methods, such as genotyping by sequencing (GBS) and high-throughput phenotyping, allowed to accelerate the cloning of QTLs associated with drought, Al, dan Fe toxicity tolerance traits (Satrio et al. 2020; Miftahudin et al. 2020). Modern rice breeding program has

been an interdisciplinary field that involves disciplines of statistics, bioinformatics, molecular biology, biochemistry, genetics, physiology, agronomy, and economics. In addition, DNA sequencing technologies have been employed to improve rice genomic and productivity. The complete rice genome sequences facilitate the development of various and robust molecular markers that can be used for genotyping and selecting large populations. Incorporating molecular breeding, high-throughput phenotyping, omics studies, and genome editing is mandatory to design the genome of drought, Al, and Fe toxicity stress tolerance rice. Genome editing with the CRISPR/Cas system is an emerging genome technology that provides simple, accurate, and precise gene-editing technology promising a new era in rice breeding and genetic improvement. Recently developed CRISPR/Cas9 technology with its variations, such as CRISPR/Cpf1, base-editing, Ribonuclease, and RGA for genome editing open new possibilities for rice genomic engineering (Yin et al. 2017).

Several negative regulators for abiotic stress tolerance could be knocked out using CRISPR technology to produce the required phenotypes for abiotic stress tolerance traits. CRISPR/Cas system has been used to develop rice lines tolerant to drought by editing *sensitive* (*S*) genes that induce drought sensitivity or negative regulators of drought stress tolerance, such as drought and salt-tolerant protein 1 (Huang et al. 2009a, b, c), drought-induced SINA protein 1 (Ning et al. 2011), and ring finger protein (Fang et al. 2015) genes. Knocking out those genes in rice produces rice tolerant to drought stress.

OsSAPK2 (a homolog of SnRK2 protein kinase) is a gene related to drought tolerance in rice. Plants expressing the OsSAPK2 gene produce OsSAPK2 protein that will phosphorylate *OsbZIP23*, a central regulator of ABA biosynthesis and signaling (Zong et al. 2016). In addition, the plants expressing the OsSAPK2 accumulate compatible solutes, increase stomata closure, and induce stress-responsive and antioxidant enzyme gene expression (Lou et al. 2017). OsSAPK2 is negatively regulated by OsPP2C49, an ABI1 homolog, that inactivates the SAPK2, hence inhibit the expression of *OsbZIP23*. Interestingly, OsbZIP23 positively regulates *OsPP2C49*, and overexpression of *OsPP2C49* in rice significantly decreased sensitivity response to abscisic acid (ABA) and induced dehydration. Knocking out the OsPP2C49 using the CRISPR/Cas system might produce rice tolerant to drought.

CRISPR/Cas system could be used for editing *cis*-acting element of abiotic stress tolerance genes, such as AREB/AFB genes by inducing epigenetic modification of drought-responsive genes to improve drought stress tolerance. AREB/ABFs serve as master transcriptional activators controlling ABRE-dependent gene expression and other downstream genes in the ABA-signaling pathway. By modifying the CRISPR/Cas9 gene-editing system with a dead Cas9 and a catalytic domain histone acetyltransferase (HAT) enzyme, Roca-Paixão et al. (2019) were able to edit *cis*-acting element of *AREB1* gene in *Arabidopsis* and obtained the drought-tolerant plant. This model might be used to modify the *cis*-acting element of AREB/AFB genes in rice to produce rice lines tolerant to drought.

Table 1.7 List of genotypes identified for Fe toxicity stress-tolerant and -moderately tolerant rice cultivars

No	Cultivar/variety	Tolerance level	References
1	Suokoko 8 (ROK24)	Tolerant	Virmani (1977), Wan et al. (2003)
2	TOX85C-C1-15-WAS 1; TOX85C-C1-16-WAS 1	Tolerant	Abifarin (1989)
3	TOX 3100-32-2-1-3-5 (WITA 3)	Tolerant	WARDA (1998)
4	TOX 3069-66-2-1-6	Tolerant	Audebert and Sahrawat (2000)
5	FKR 19	Tolerant	Ouedraogo and Ouedraogo (2003)
6	TOX 3100-44-1-2-3-3 (WITA 4); TOX 4216-25-2-3-1-3; WAT 1059-B-51-2; WAT 1282-B-3-3; WAT 1131-B-26-2-1-2	Tolerant	Gridley et al. (2006)
7	CK 4; CK 73	Tolerant	Abdoul (2006)
8	BW 348-1	Tolerant	Aboa and Dogbe (2006)
9	Nerica-L19	Tolerant	Dramé et al. (2010)
10	IPB Kapuas 7R; IPB Batola 6R; IPB1 R Dadahub; IPB Batola 5R; Indragiri; Margasari; A Tenggulang	Tolerant	Nugraha and Rumanti (2017)
11	Kapuas	Tolerant	Suhartini et al. (1996)
12	Inpara 2; B13144-1; Cilamaya Muncul; Margasari	Tolerant	Nugraha et al. (2016)
13	B13144-1-MR-2	Tolerant	Suhartini and Makarim (2009)
14	Cilamaya, Siam Saba, Mahsuri, Pokkali, Awan Kuning	Tolerant	Nugraha et al. (2016)
15	IR61246-3B-15-2-2-3; IR61612-3B-16-2-2-1; IR61640-3B-14-3-3-2; WITA 7; Suokoko 8 (ROK 24); TCA 4; Azucena	Tolerant	Elec et al. (2013)
16	CK4; Tox4004-8-1-2-3	Tolerant	Asch et al. (2005)
17	OG 7206; TOG 6218-B; TOG 7250-A	Tolerant	Sikirou et al. (2016)
18	BR IRGA 4141; IRGA 419; BRS AGRISUL	Tolerant	Crestani et al. (2009)
19	ISA-40; PSQ-4	Tolerant	Ramírez et al. (2002)
20	EPAGRI 108	Tolerant	Da silveira et al. (2007)
21	Dom Sofid	Tolerant	Frei et al. (2016)

(continued)

Table 1.7 (continued)

No	Cultivar/variety	Tolerance level	References
22	Mahsurian	Moderately tolerant	Suhartini and Makarim (2009)
23	WITA1; Matkandu	Moderately tolerant	Audebert and Fofana (2009)
24	Inpara 3	Moderately tolerant	Nugraha et al. (2016)
25	CG14; I Kong Pao; Sahel 108; ITA 306; ITA 320	Moderately tolerant	Becker and Asch (2005)
26	IR74; Mahsuri	Moderately tolerant	Wan et al. (2003)

Editing the genes responsible for several traits related to drought tolerance has also been conducted using the CRISPR/Cas system to produce mutant rice that tolerant to drought stress. *Semi-rolled leaf1* (SRL1) and SRL2 are the genes that involve in leaf rolling through controlling the number, size, and arrangement of a bulliform cell (Liu et al. 2016). Rolled leaf mutant rice plants have been developed through editing the *SRL1* and *SRL2* genes using the CRISPR/Cas9 gene-editing system (Liao et al. 2019). The mutant showed a higher tolerant level to drought stress compare to the wild type. The mutants have good morpho-physiological and agronomic characters related to drought tolerance parameters, such as semi-rolled leaves; low number and conductance of stomata; low transpiration rate, chlorophyll content, and vascular bundles; high ABA content, superoxide dismutase, and catalase activities; a high number of panicle and grain; and high grain filling and yield per plant.

Development of rice tolerant to abiotic stresses also includes tolerance to Al and Fe toxicity. Many genes involved in Al and Fe tolerant have been cloned and characterized. One of the genes that responsible for Al tolerance in rice is OsFRDL4, a gene encoding citrate transporter in rice. The gene is regulated by ART1 and WRKY22 transcription factors. Knocking out the WRKY22 gene to induce mutation to impair the function of OsFRDL4 to secret citrate when exposed to Al stress (Li et al. 2018a, b). Modifying the cis-acting element of either WRKY22 or OsFRDL4 gene through CRISPR/Cas based gene editing to overexpressed the gene might produce rice plant secrete more citrate during Al stress, hence make the plant tolerant to Al stress.

Genes for *ferritin*, *VIT*, *NAS3*, *HRZ*, WRKYs, and *GSNOR* are suggested to involve in plant tolerance to Fe excess (Aung and Masuda 2020). Li et al. (2019) showed that the *GSNOR* gene is involved in tolerance to Fe toxicity via nitric oxide (NO) pathway. Another research showed that an iron transporter gene (*OsIRO3*) has a relationship to the ability of a plant to prevent Fe excess in a plant through regulation of signal transmission from shoots to roots (Wang et al. 2020a, b). The roles of *GSNOR* and *OsIRO3* genes in rice plant were revealed by using knocked

out mutant using CRISPR/Cas9. In the future, the *OsIRO3* gene could be one of the promising candidate genes that can be used for improving the Fe toxicity tolerance in rice.

1.6 Future Perspective

Tolerance to abiotic stresses in rice is a complex trait involving many genes that are expressed to various abiotic stress-related phenotypes. The designed genome for genotype and phenotype improvement of rice plant tolerance to drought stress should consider the combining phenotype in one individual plant, such as pyramiding the trait to produce rice plants tolerant of multi abiotic stresses. CRISPR/Cas system with its variation is a promising genome editing technique that can be used to develop rice varieties with the pre-designed genome. Multiple gene editing could be carried out in one plant to target various genes or regulators responsible for multi-stress abiotic tolerance. A CRISPR/Cas vector construct containing an array of sgRNA developed from different abiotic stress-related genes can be developed to target multisite in the rice genome. More accurate CRISPR/Cas, such as CRISPR/Cas12a system might be used to target multi-editing site with more than one endogenous gene targets in order to develop a rice plant tolerant to multi abiotic stress.

Application of the existing CRISPR/Cas-based gene-editing techniques in rice improvement can be effectively improved by combining the technique with speed breeding. The genome-edited transgenic rice can be grown under speed breeding conditions to produce mutant seeds in a short generation time. Using this method, the time to achieve stable homozygous phenotypes will be faster than conventional breeding of GMO development. The limitation in the regeneration of transgenic plants, especially for recalcitrant rice genotypes, is a constraint that needs to be solved with developed tissue culture technology. In the future, the CRISPR/Cas9 system to edit genome combined with speed breeding will likely be the main alternative to design genome in rice breeding program for abiotic stress tolerance.

References

Abdoul KC (2006) Testing and developing tolerant rice varieties to iron toxicity in lower guinea (CRA Kilissi and Koba). In: Audebert A, Narteh LT, Kiepe P, Millar D, Beks B (eds) Iron toxicity in rice-based system in West Africa. WARDA, Cotonou

Abifarin AO (1989) Progress in breeding rice for tolerance to iron toxicity. In: WARDA (ed) WARDA annual report for 1990. West Africa Rice Development Association, Bouaké

Aboa K, Dogbe SY (2006) Effect of iron toxicity on rice yield in the Amou-Oblo lowland in Togo. In: Audebert A, Narteh LT, Kiepe P, Millar D, Beks B (eds) Iron toxicity in rice-based system in West Africa. WARDA, Cotonou

Adiningsih J, Sudjadi M (1993) The role of alley cropping systems in increasing soil fertility on acid dryland. Bogor: Indonesian Center for Soil and Agro-climate Research and Development [in Indonesian]

Adjao RT, Staatz JM (2015) Asian rice economy changes and implications for Sub-Sharan Africa. Glob Food Secur 5:50–55

Ahmed CB, Rouina BB, Sensoy S, Boukhris M, Abdallah FB (2009) Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. Environ Exp Bot 67:345–352

Ali M, Pathan MS, Zhang J, Bai G, Sarkarung S, Nguyen HT (2000) Mapping QTLs for root traits in a recombinant inbred population from two indica ecotypes in rice. Theor Appl Genet 101:756–766

Ali M, McClung AM, Jia MH, Kimball JA, McCouch SR, Eizenga GC (2011) Rice diversity panel evaluated for genetic and agro-morphological diversity between subpopulations and its geographic distribution. Crop Sci 51:2021–2035

Aniol A, Gustafson JP (1984) Chromosome location of genes controlling aluminum tolerance in wheat, rye and triticale. Can J Genet Cytol 26:701–705

Anjum SA, Xie X, Wang L (2011) Morphological, physiological and biochemical responses of plants to drought stress. Afr J Agric Res 6:2026–2032

Arenhart RA, Yang B, Oliveira LF, Neto LB, Schunemann M, Maraschin FS, Margis-Pinheiro M (2014) New insights into aluminum tolerance in rice: the ASR5 protein binds the *STAR1* promoter and other aluminum-responsive genes. Mol Plant 7:709–721

Arenhart RA, Margis R, Margis-Pinheiro M (2016) Rice ASR1 and ASR5 are complementary transcription factors regulating aluminium responsive genes. Plant Cell Environ 39:645–651

Arunin S, Hillerislambers D (1984) Rice cultivation in the tidal swamps of Thailand. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June 1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 79–88

Asch F, Becker M, Kpongor DS (2005) A quick and efficient screen for resistance to iron toxicity in lowland rice. J Plant Nutr Soil Sci 168(6):764–773

Atwell S, Huang YS, Vilhjalmsson BJ, Willems G, Horton M, Li Y, Meng D, Platt A, Tarone AM, Hu TT, Jiang R, Muliyati NW, Zhang X, Amer MA, Baxter I, Brachi B, Chory J, Dean C, Debieu M, de Meaux J, Ecker JR, Faure N, Kniskern JM, Jones JD, Michael T, Nemri A, Roux F, Salt DE, Tang C, Todesco M, Traw MB, Weigel D, Marjoram P, Borevitz JO, Bergelson J, Nordborg M (2010) Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. Nature 465:627–631

Audebert A (2006) Iron toxicity in rice—environmental conditions and symptoms. In: Audebert A, Narteh LT, Kiepe P, Millar D, Beks B (eds) Iron toxicity in rice-based system in West Africa. WARDA, Cotonou

Audebert A, Sahrawat KL (2000) Mechanisms for iron toxicity tolerance in lowland rice. J Plant Nutr Soil Sci 23(11–12):1877–1885

Audebert A, Fofana M (2009) Rice yield gap due to iron toxicity in West Africa. J Agron Crop Sci 195(1):66–76

Aung MS, Masuda H (2020) How does rice defend against excess iron? Physiological and molecular mechanisms. Front Plant Sci 11:1102

Aung MS, Masuda H, Kobayashi T, Nishizawa NK (2018) Physiological and transcriptomic analysis of responses to different levels of iron excess stress in various rice tissues. J Soil Sci Plant Nutr 64(3):370–385

Babu RC, Zhang J, Blum A, Ho THD, Wu R, Nguyen HT (2004a) HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. Plant Sci 166:855–862

Babu RC, Zhang J, Blum A, Ho THD, Wu R, Nguyen HT (2004b) HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. Plant Sci 116:855–862

Bae H, Kim SK, Cho SK, Kang BG, Kim WT (2011) Overexpression of OsRDCP1, a rice RING domain-containing E3 ubiquitin ligase, increased tolerance to drought stress in rice (*Oryza sativa* L.). Plant Sci 180:775–782

Barik SR, Pandit E, Pradhan SK, Mohanty SP, Mohapatra T (2019) Genetic mapping of morpho-physiological traits involved during reproductive stage drought tolerance in rice. PLoS ONE 14:e0214979

Barik SR, Pandit E, Mohanty SP, Nayak DK, Pradhan SK (2020) Genetic mapping of physiological traits associated with terminal stage drought tolerance in rice. BMC Genet 76:1471–2156

Bashir K, Nozoye T, Ishimaru Y, Nakanishi H, Nishizawa NK (2013) Exploiting new tools for iron bio-fortification of rice. Biotech Adv 31(8):1624–1633

Bauer H, Ache P, Lautner S, Fromm J, Hartung W, Al-Rasheid KAS et al (2013) The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. Curr Biol 23:53–57

Becker M, Asch F (2005) Iron toxicity in rice: conditions and management concepts. J Plant Nutr Soil Sci 168:558–573

Behnam B, Iuchi S, Fujita M, Fujita Y, Takasaki H, Osakabe Y et al (2013) Characterization of the promoter region of an *Arabidopsis* gene for 9-cis-epoxycarotenoid dioxygenase involved in dehydration-inducible transcription. DNA Res 20:315–324

Bernier J, Kumar A, Ramaiah V, Spaner D, Atlin GN (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Sci 47:507–518

Bian M, Zhou M, Sun D, Li C (2013) Molecular approaches unravel the mechanism of acid soil tolerance in plants. Crop J 1(2):91–104

Bihani P, Char B, Bhargava S (2011) Transgenic expression of sorghum DREB2 in rice improves tolerance and yield under water limitation. J Agr Sci 149:95–101

Briat JF, Lobréaux S (1997) Iron transport and storage in plants. Trends Plant Sci 2(5):187–193

Brumbarova T, Bauer P (2008) Plant membrane and vacuolar transporters. CAB International, London

Budzianowski G, Wos H (2004) The effect of single D-genome chromosomes on aluminum tolerance of triticale. Euphytica 137:165–172

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Burr B, Burr FA, Thompson KH, Albertson MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Byrne A, Cole C, Volden R, Vollmers C (2019) Realizing the potential of full-length transcriptome sequencing. Phil Trans Roy Soc B 374:20190097

Campo S, Peris-Peris C, Montesinos L, Peñas G, Messeguer J, San Segundo B (2012) Expression of the maize ZmGF14-6 gene in rice confers tolerance to drought stress while enhancing susceptibility to pathogen infection. J Exp Bot 63:983–999

Campo S, Baldrich P, Messeguer J, Lalanne E, Coca M, San Segundo B (2014) Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. Plant Physiol 165:688–704

Caniato F, Guimaraes C, Schaffert R, Alves V, Kochian LV (2007) Genetic diversity for aluminum tolerance in sorghum. Theor Appl Genet 114:863–876

Capell T, Escobar C, Liu H, Burtin D, Lepri O, Christou P (1998) Over-expression of the oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development patterns *in vitro* and results in putrescine accumulation in transgenic plants. Theor Appl Genet 97:246–254

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

Carew BP (1984) Large-scale farming bordering the Musi River tidal swamps: the P. T. Patra Tani Project. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June 1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 29–36

Chatterjee C, Gopal R, Dube BK (2006) Impact of iron stress on biomass, yield, metabolism and quality of potato (*Solanum tuberosum* L.). Sci Hort 108(1):1–6

Che J, Tsutsui T, Yokosho K, Yamaji N, Ma JF (2018) Functional characterization of an aluminum (Al)-inducibletranscription factor, ART2, revealed a different pathway for Altolerance in rice. New Phytol 18:1–10

Chen JQ, Meng XP, 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 LS, Qi YP, Jiang HX, Yang LT, Yang GH (2010) Photosynthesis and photoprotective systems of plants in response to aluminum toxicity. Afr J Biotechnol 9:9237–9247

Chen ZC, Yamaji N, Motoyama R, Nagamura Y, Ma JF (2012a) Up-regulation of a magnesium transporter gene *OsMGT1* is required forconferring aluminum tolerance in rice. Plant Physiol 159:1624–1633

Chen H, Chen W, Zhou J, He H, Chen L, Chen H et al (2012b) Basic leucine zipper transcription factor OsbZIP16 positively regulates drought resistance in rice. Plant Sci 193–194:8–17

Ching A, Caldwell K, Jung M, Dolan M, Smith O, Tingey S, Morgante M, Rafalski AJ (2002) SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genet 3:1–19

Conte SS, Walker EL (2011) Transporters contributing to iron trafficking in plants. Mol Plant 4 (3):464–476

Crestani M, Da Silva JAG, de Souza VQ, Hartwig I, de Souza Luche H, de Sousa RO, De Carvalho FIF, De Oliveira AC, Luche HDS, de Sousa RO, de Carvalho FIF, de Oliveira AC (2009) Irrigated rice genotype performance under excess iron stress in hydroponic culture. Crop Breed Appl Biotechnol 9:85–93

Cui M, Zhang W, Zhang Q, Xu Z, Zhu Z, Duan F et al (2011) Induced over-expression of the transcription factor OsDREB2A improves drought tolerance in rice. Plant Physiol Biochem 49:1384–1391

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651–679

Da Silveira VC, de Oliveira AP, Sperotto RA, Espindola LS, Amaral L, Dias JF, da Cunha JB, Fett JP (2007) Influence of iron on mineral status of two rice (*Oryza sativa* L.) cultivars. Braz J Plant Physiol 19(2):127–139

Daly M, Rioux J, Schaffner S, Hudson T, Lander E (2001) High-resolution haplotype structure in the human genome. Nat Genet 29:229–232

Datta K, Baisakh N, Ganguly M, Krishnan S, Yamaguchi-Shinozaki K, Datta SK (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 Oliveira Jucoski G, Cambraia J, Ribeiro C, de Oliveira JA, de Paula SO, Oliva MA (2013) Impact of iron toxicity on oxidative metabolism in young *Eugenia uniflora* L. plants. Acta Physiol Plant 35:1645–1657

Delhaize E, Ryan PR, Randall PJ (1993) Aluminum tolerance in wheat (*Triticum aestivum* L.): II. Aluminum stimulated excretion of malic acid from rootapices. Plant Physiol 103:695–702

Delhaize E, Hebb DM, Ryan PR (2001) Expression of *Pseudomonas aeruginosacitrate* synthase gene in tobacco is not associated with either enhanced citrate accumulation of efflux. Plant Physiol 125:2059–2067

Devi AG, Yadav GS, Devi HL, Ngachan SV (2016) Effect of acute iron toxicity on key antioxidative enzymes in contrasting rice (*Oryza sativa* L.) cultivars of North-East India. IJBSM 7(3):388–392

Ding ZJ, Yan JY, Xu XY, Li GX, Zheng SJ (2013) WRKY46 functions as a transcriptional repressor of *ALMT1*, regulating aluminum-induced malate secretion in *Arabidopsis*. Plant J 76:825–835

Dixit S, Swamy BPM, Vikram P, Ahmed HU, Sta Cruz MT, Amante M, Atri D, Leung H, Kumar A (2012) Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. Theor Appl Genet 125:155–169

Dobermann A, Fairhurst T (2000) Mineral toxicities. In: Dobermann A, Fairhurst A (eds) Rice: nutrient disorders & nutrient management. Potash & Phosphate Institute (PPI), Potash & Phosphate Institute of Canada (PPIC) and International Rice Research Institute

Dramé KN, Saito K, Koné B, Chabi A, Dakouo D, Annan-Afful E, Monh S, Abo E, Sié M (2010) Coping with iron toxicity in the lowlands of sub-Saharan Africa: experience from Africa rice center. In: AfricaRice (ed) Innovation and partnerships to realize Africa's rice potential. Proceedings of 2nd Africa rice congress, 22–26 March 2010, Bamako

Du H, Wang N, Cui F, Li X, Xiao J, Xiong L (2010) Characterization of a β-carotene hydroxylase gene DSM2 conferring drought and oxidative stress resistance by increasing xanthophylls and ABA synthesis in rice. Plant Physiol 154:1304–1318

Duan J, Cai W (2012) OsLEA3-2, an abiotic stress induced gene of rice plays a key role in salt and drought tolerance. PLoS ONE 7:e45117

Dufey I, Hakizimana P, Draye X, Lutts S, Bertin P (2009) QTL mapping for biomass and physiological parameters linked to resistance mechanisms to ferrous iron toxicity in rice. Euphytica 167:143–160

Dufey I, Hiel MP, Hakizimana P, Draye X, Lutts S, Koné B, Dramé KN, Konaté KA, Sie M, Bertin P (2012) Multienvironment quantitative trait loci mapping and consistency across environments of resistance mechanisms to ferrous iron toxicity in rice. Crop Sci 52(2):539–550

Dufey I, Mathieu AS, Draye X, Lutts S, Bertin P (2015a) Construction of an integrated map through comparative studies allows the identification of candidate regions for resistance to ferrous iron toxicity in rice. Euphytica 203:59–69

Dufey I, Draye X, Lutts S, Lorieux M, Martinez C, Bertin P (2015b) Novel QTLs in an interspecific backcross *Oryza sativa* × *Oryza glaberrima* for resistance to iron toxicity in rice. Euphytica 204:609–625

Elec V, Quimio CA, Mendoza R, Sajise AGC, Beebout SEJ, Gregorio GB, Singh RK (2013) Maintaining elevated Fe^{2+} concentration in solution culture for the development of a rapid and repeatable screening technique for iron toxicity tolerance in rice (*Oryza sativa* L.). Plant Soil 372(1):253–264

Engel K, Asch F, Becker M (2012) Classification of rice genotypes based on their mechanisms of adaptation to iron toxicity. J Plant Nutr Soil Sci 175(6):871–881

Ereful NC, Liu L, Greenland A, Powell W, Mackay I, Leung H (2020) RNA-seq reveals differentially expressed genes between two Indica inbred rice genotypes associated with droughty yield QTLs. Agronomy 10:1–19

Fahramand M, Mahmoody M, Keykha A, Noori M, Rigi K (2014) Influence of abiotic stress on proline, photosynthetic enzymes and growth. Intl Res J Appl Basic Sci 8:257–265

Famoso A, Clark R, Shaff J, Craft E, McCouch S (2010) Development of a novel aluminum tolerance phenotyping platform used for comparisons of cereal aluminum tolerance and investigations into rice aluminum tolerance mechanisms. Plant Physiol 153:1678

Famoso AN, Zhao K, Clark RT, Tung CW, Wright MH, Bustamante C, Kochian LV, McCouch SR (2011) Genetic architecture of aluminum tolerance in rice (*Oryza sativa*) determined through genome-wide association analysis and QTL mapping. PLoS Genetics 7:1–16

Fang H, Meng Q, Xu J, Tang H, Tang S, Zhang H, Huang J (2015) Knock-down of stress inducible OsSRFP1 encoding an E3 ubiquitin ligase with transcriptional activation activity confers abiotic stress tolerance through enhancing antioxidant protection in rice. Plant Mol Biol 87:441–458

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212

Fendiyanto MH, Satrio RD, Suharsono S, Tjahjoleksono A, Miftahudin M (2019a) Correlation among Snpb11 markers, root growth, and physiological characters of upland rice under aluminum stress. Biodiversitas 20:1243–1254

Fendiyanto MH, Satrio RD, Suharsono S, Tjahjoleksono A, Miftahudin M (2019b) QTL for aluminum tolerance on rice chromosome-3 based on root length characters. SABRAO J Breed Genet 51:451–469

Finatto T, de Oliveira AC, Chaparro C, da Maia LC, Farias DR, Woyann LG, Mistura CC, Soares-Bresolin AP, Llauro C, Panaud O, Picault N (2015) Abiotic stress and genome dynamics: specific genes and transposable elements response to iron excess in rice. Rice 8 (13):1–18

Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Annu Rev Plant Physiol 29:51l–566

Frei M, Tetteh RN, Razafindrazaka AL, Fuh MA, Wu LB, Becker M (2016) Responses of rice to chronic and acute iron toxicity: genotypic differences and biofortification aspects. Plant Soil 408:149–161

Fukuda A, Shiratsuchi H, Fukushima A, Yamaguchi H, Mochida H, Terao T, Ogiwara H (2012) Detection of chromosomal regions affecting iron concentration in rice shoots subjected to excess ferrous iron using chromosomal segment substitution lines between *Japonica* and *Indica*. Plant Prod Sci 15(3):183–191

Furukawa J, Yamaji N, Wang H, Mitani N, Murata Y (2007) An aluminum-activated citrate transporter in barley. Plant Cell Physiol 48:1081–1087

Ganal MW, Polley A, Graner EM, Plieske J, Wieseke R, Luerssen H, Durstewitz G (2012) Large SNP arrays for genotyping in crop plants. J Biosci 37:821–828

Gao T, Wu Y, Zhang Y, Liu L, Ning Y, Wang D, Xie Q (2011) OsSDIR1 overexpression greatly improves drought tolerance in transgenic rice. Plant Mol Biol 76:145–156

Garris AJ, McCouch SR, Kresovich S (2003) Population structure and its effect on haplotype diversity and linkage disequilibrium surrounding the *xa5* locus of rice (*Oryza sativa* L.). Genetics 165:759–769

Ghimire KH, Quiatchon LA, Vikram P, Swamy BPM, Dixit S, Ahmed HU, Hernandez JE, Borromeo TH, Kumar A (2012) Identification and mapping of a QTL (qDTY1.1) with a consistent effect on grain yield under drought. Field Crops Res 131:88–96

Gomez SM, Boopathi NM, Kumar SS, Ramasubramanian T, Chengsong Z, Jeyaprakash P, Senthil A, Babu RC (2010) Molecular mapping and location of QTLs for drought-resistance traits in indica rice (*Oryza sativa* L.) lines adapted to target environments. Acta Physiol Plant 32:355–364

Gridley HE, Efisue A, Tolou B, Bakayako T (2006) Breeding for tolerance to iron toxicity at WARDA. In: Audebert A, Narteh LT, Kiepe P, Millar D, Beks B (eds) Iron toxicity in rice based system in West Africa. WARDA, Cotonou

Guerinot ML, Ying Y (1994) Iron: nutritious, noxious, and not readily available. Plant Physiol 104 (3):815–820

Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF et al (2010) Significant acidification in major Chinese croplands. Science 327:1008–1010

Guo P, Qi YP, Cai YT, Yang TY, Yang LT, Huang ZR et al (2018) Aluminum effects on photosynthesis, reactive oxygen species and methylglyoxal detoxification in two Citrus species differing in aluminum tolerance. Tree Physiol 38:1548–1565

Hamid MA, Islam MR (1984) Rice cultivation in the tidal swamps of Bangladesh. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June 1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 69–78

Harushima Y, Yano M, Shomura A et al (1998) A high-density rice genetic linkage map with 2275 markers using a single F2 population. Genetics 148:479–494

Hayes M, Svoboda M, Wall N, Widhalm M (2010) The Lincoln declaration on drought indices: universal meteorological drought index recommended. Bull Amer Meteorol Soc 92:485–488

Hemamalini GS, Shashidhar HE, Hittalmani S (2000) Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice (*Oryza sativa* L.). Euphytica 112:69–78

Ho SL, Huang LF, Lu CA, He SL, Wang CC, Yu SP et al (2013) Sugar starvation- and GA-inducible calcium-dependent protein kinase 1 feedback regulates GA biosynthesis and activates a 14-3-3 protein to confer drought tolerance in rice seedlings. Plant Mol Biol 81:347–361

Hoekenga O, Vision T, Shaff J, Monforte A, Lee G, Howell SH, Kochian LV (2003) Identification and characterization of aluminum tolerance loci in *Arabidopsis* (*Landsberg erecta* × Columbia) by quantitative trait locus mapping. A physiologically simple but genetically complex trait. Plant Physiol 132:930–936

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q et al (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

Huang C, Yamaji N, Mitani N, Yano M, Nagamura Y, Ma JF (2009a) A bacterial-type ABC transporter is involved in aluminum tolerance in rice. Plant Cell 21:655–667

Huang XY, Chao DY, Gao JP, Zhu MZ, Shi M, Lin HX (2009b) A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Genes Dev 23:1805–1817

Huang J, Sun SJ, Xu DQ, Yang X, Bao YM, Wang ZF et al (2009c) Increased tolerance of rice to cold, drought and oxidative stresses mediated by the over expression of a gene that encodes the zinc finger protein ZFP245. Biochem Biophys Res Commun 389:556–561

Huang C, Yamaji N, Chen Z, Ma JF (2012a) A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice. Plant Cell 69:857–867

Huang J, Sun S, Xu D, Lan H, Sun H, Wang Z et al (2012b) A TFIIIA-type zinc finger protein confers multiple abiotic stress tolerances in transgenic rice (*Oryza sativa* L.). Plant Mol Biol 80:337–350

Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y, Li C, Zhu C, Lu T, Zhang Z, Li M, Fan D, Guo Y, Wang A, Wang L, Deng L, Li W, Lu Y, Weng Q, Liu K, Huang T, Zhou T, Jing Y, Li W, Lin Z, Buckler ES, Qian Q, Zhang QF, Li J, Han B (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet 42:926–927

Inoue H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y, Suzuki K, Nakazono M, Nakanishi H, Mori S, Nishizawa NK (2009) Rice *OsYSL15* is an iron-regulated iron (III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. J Biol Chem 284(6):3470–3479

IRRI (2006) Bringing hope, improving lives-strategies. Plan 2007–2015. International Rice Research Institute, Los Banos, p 61

Ishimaru Y, Suzuki M, Tsukamoto T, Suzuki K, Nakazono M, Kobayashi T, Wada Y, Watanabe S, Matsuhashi S, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2006) Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. Plant J 45(3):335–346

Ishizaki T, Maruyama K, Obara M, Fukutani A, Yamaguchi-Shinozaki K, Ito Y et al (2012) Expression of *Arabidopsis* DREB1C improves survival, growth, and yield of upland new rice for Africa (NERICA) under drought. Mol Breed 31:255–264

Ismunadji M, Partohardjono S (1985) Program hasil penelitian pengapuran tanah masamuntuk peningkatan produksi tanaman pangan. Balai Penelitian Tanah, Bogor

Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, 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

Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T et al (2001) Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. Plant J 27:325–333

Iuchi S, Koyama H, Iuchi A, Kobayashi Y, Kitabayashi S (2007) Zinc finger protein STOP1 is critical for proton tolerance in *Arabidopsis* and coregulates a key gene in aluminum tolerance. Proc Natl Acad Sci USA 104:9900–9905

Jang IC, Oh SJ, Seo JS, Choi WB, Song SI, Kim CH, Kim JK (2003) Expression of a bifunctional fusion of the *Escherichia coli* genes for trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase in transgenic rice plants increases trehalose accumulation and abiotic stress tolerance without stunting growth. Plant Physiol 131:516–524

Jayawardena SDG (1984) Rice cultivation in the tidal swamps of Sri Lanka. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June

1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 107–114

Jeffreys A, Kauppi L, Neumann R (2001) Intensely punctate meiotic recombination in the class II region of the major histocompatibility complex. Nat Genet 29:217–222

Jeong J, Connolly EL (2009) Iron uptake mechanisms in plants: functions of the FRO family of ferric reductases. Plant Sci 176(6):709–714

Jeong JS, Kim YS, Baek KH, Jung H, Ha SH, Do Choi Y et al (2010) Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. Plant Physiol 153:185–197

Jeong JS, Kim YS, Redillas MCFR, Jang G, Jung H, Bang SW et al (2013) OsNAC5 over expression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. Plant Biotechnol J 11:101–114

Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M, Agier N, Bulski A, Albuisson J, Heredia F, Audigier P, Bouchez D, Dillmann C, Guerche P, Hospital F, Colot V (2009) Assessingthe impact of transgenerational epigenetic variation oncomplex traits. PLoS Genet 5:100–105

Johnson L, Andrade F, Salvador JC, Penafiel W, Vergara BS (1984) Rice cultivation in the tidal swamps of Samborondon, Ecuador. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June 1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 89–106

Joo J, Choi HJ, Lee YH, Kim YK, Song SI (2013) A transcriptional repressor of the ERF family confers drought tolerance to rice and regulates genes preferentially located on chromosome 11. Planta 238:155–170

Jumiati (2016) Physiological characteristics of aluminum sensitive mutant-rice roots and their inheritance patterns. IPB Press, Bogor

Jung M, Ching A, Bhattramakki D, Dolan M, Tingey S, Morgante M, Rafalski A (2004) Linkage disequilibrium and sequence diversity in a 500-kbp region around the adh1 locus in elite maize germplasm. Theor Appl Genet 109:681–689

Kang J, Choi H, Im M, Kim SY (2002) *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell 14:343–357

Kanneganti V, Gupta AK (2008) Overexpression of OsiSAP8, a member of stress associated protein (SAP) gene family of rice confers tolerance to salt, drought and cold stress in transgenic tobacco and rice. Plant Mol Biol 66:445–462

Karaba A, Dixit S, Greco R, Aharoni A, Trijatmiko KR, Marsch-Martinez N et al (2007) Improvement of water use efficiency in rice by expression of HARDY, an *Arabidopsis* drought and salt tolerance gene. Proc Natl Acad Sci USA 104:15270–15275

Kasuga K, Liu Q, Miura S, Yamaguchi-Shinozaki K, 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

Kawahara Y, de la Bastide M, Hamilton JP, Kanamori H, McCombie WR (2013) Improvement of the *Oryza sativa* nipponbare reference genome using next generation sequence and optical map data. Rice 6:4

Khowaja FS, Norton GJ, Courtois B, Price AH (2009) Improved resolution in the position of drought-related QTLs in a single mapping population of rice by meta-analysis. BMC Genom 10:276

Kidd PS, Lugany M, Poschenrieder C, Gunse B, Barcelo J (2001) The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). J Exp Bot 52:1339–1352

Kim S, Plagnol V, Hu TT, Toomajian C, Clark RM, Ossowski S, Ecker JR, Weigel D, Nordborg M (2007) Recombination and linkage disequilibrium in *Arabidopsis thaliana*. Nat Genet 39:1151–1155

Kim H, Hwang H, Hong JW, Lee YN, Ahn IP, Yoon IS et al (2012) A rice orthologue of the ABA receptor, OsPYL/RCAR5, is a positive regulator of the ABA signal transduction pathway in seed germination and early seedling growth. J Exp Bot 63:1013–1024

Kim H, Lee K, Hwang H, Bhatnagar N, Kim DY, Yoon IS et al (2014) Over expression of PYL5 in rice enhances drought tolerance, inhibits growth, and modulates gene expression. J Exp Bot 65:453–464

Kinraide TB (1991) Identity of the rhizotoxicaluminium species. Plant Soil 134:167–178

Kobayashi T, Nishizawa NK (2012) Iron uptake, translocation, and regulation inhigher plants. Annu Rev Plant Biol 63:131–152

Kobayashi Y, Ohyama Y, Kobayashi Y, Ito H, Iuchi S (2014) STOP2 activates transcription of several genes for Al- and low pH-tolerance that are regulated by STOP1 in *Arabidopsis*. Mol Plant 7:311–322

Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Annu Rev Plant Physiol Plant Mol Biol 46:237–260

Kochian LV (2000) Molecular physiology of mineral nutrient acquisition, transport and utilization. American Society of Plant Physiologists, Maryland

Kochian LV, Hoekenga OA, Piñeros MA (2004) How do crop plants tolerant acid soils? Mechanisms of aluminum toxicity and phosphorus efficiency. Annu Rev Plant Biol 55:459–493

Kochian LV, Pineros MA, Hoekenga OA (2005) The physiology, genetics and molecular biology of plant aluminum tolerance and toxicity. Plant Soil 274:175–195

Kochian LV, Piñeros MA, Liu J, Magalhaes JV (2015) Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. Annu Rev Plant Biol 66:1–28

Koskeroglu S, Tuna AL (2010) The investigation on accumulation levels of proline and stress parameters of the maize (*Zea mays* L.) plants under salt and water stress. Acta Physiol Plant 32:541–549

Koswara O, Rumawas F (1984) Tidal swamp rice in Palembang region. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June 1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 37–48

Krohling CA, Eutrópio FJ, Bertolazi AA, Dobbss LB, Campostrini E, Dias T, Ramos AC (2016) Ecophysiology of iron homeostasis in plants. Soil Sci Plant Nutr 62(1):39–47

Kumar R, Venuprasad R, Atlin GN (2007) Genetic analysis of rain-fed lowland rice drought tolerance under naturally-occurring stress in eastern India: heritability and QTL effects. Field Crops Res 103:42–52

Kumar VVS, Verma RK, Yadav SK, Yadav P, Watts A, Rao MV, Chinnusamy V (2020) CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. Physiol Mol Biol Plants 26:1099–1110

Kuromori T, Sugimoto E, Shinozaki K (2014) Intertissue signal transfer of abscisic acid from vascular cells to guard cells. Plant Physiol 164:1587–1592

Lafitte HR, Courtois B, Arraudeau M (2002) Genetic improvement of rice in aerobic systems: progress from yield to genes. Field Crops Res 75:2–3

Lafitte HR, Price AH, Courtois B (2004) Yield response to water deficit in an upland rice mapping population: associations among traits and genetic markers. Theor Appl Genet 109:1237–1246

Lanceras CJ, Pantuwan G, Jongdee B, Toojinda T (2004) Quantitative trait loci associated with drought tolerance at reproductive stage in rice. Plant Physiol 135:384–399

Larsen PB, Geisler MJB, Jones CA, Williams KM, Cancel JD (2005) *ALS3* encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in *Arabidopsis*. Plant J 41(3):353–363

Larsen PB, Cancel J, Rounds M, Ochoa V (2007) *Arabidopsis ALS1* encodes a root tip and stele localized half type ABC transporter required for root growth in an aluminum toxic environment. Planta 225(6):1447–1458

Lenka S, Katiyar A, Chinnusamy V, Bansal K (2011) Comparative analysis of drought-responsive transcriptome in Indica rice genotypes with contrasting drought tolerance. Plant Biotechnol J 9:315–327

Li Xf, Ma JF, Matsumoto H (2000) Pattern of aluminum-induced secretion oforganic acids differs between *rye* and wheat. Plant Physiol 123:1537–1543

Li Z, Mu P, Li C, Zhang H, Li Z, Gao Y, Wang X (2005) QTL mapping of root traits in a doubled haploid population from a cross between upland and lowland japonica rice in three environments. Theor Appl Genet 110:1244–1252

Lynch JP, Saint-Clair SB (2004) Mineral stress: the missing link in understanding how global climate change will affect plants in real world soils. Field Crops Res 90(1):101–115

Li HW, Zang BS, Deng XW, Wang XP (2011) Overexpression of the trehalose-6-phosphate synthase gene OsTPS1 enhances abiotic stress tolerance in rice. Planta 234:1007–1008

Li JY, Liu J, Dong D, Jia X, McCouch SR, Kochian LV (2014) Natural variation underlies alterations in Nramp aluminum transporter (*NRAT1*) expression and function that play a key role in rice aluminum tolerance. Proc Natl Acad Sci USA 111:6503–6518

Li GZ, Wang ZQ, Yokosho K, Ding B, Fan W, Gong QQ, Li GX, Wu YR, Yang JL, Ma JF, Zheng SJ (2018a) Transcription factor WRKY22 promotes aluminum tolerance via activation of OsFRDL4 expression and enhancement of citrate secretion in rice (*Oryza sativa*). New Phytol 219:149–162

Li Y, Xiao J, Chen L, Huang X, Cheng Z, Han B, Zhang Q, Wu C (2018b) Rice functional genomics research: past decade and future. Mol Plant 11:359–380

Li B, Sun L, Huang J, Gösch C, Shi W, Chory J, Busch W (2019) GSNOR provides plant tolerance to iron toxicity via preventing iron-dependent nitrosative and oxidative cytotoxicity. Nat Commun 10(1):3896

Liang X, Zhang L, Natarajan SK, Becker DF (2013) Proline mechanisms of stress survival. Antioxid Redox Signal 19:998–1011

Liao S, Qin X, Luo L, Han Y, Wang X, Usman B et al (2019) CRISPR/Cas9-induced mutagenesis of semi-rolled leaf 1, 2 confers curled leaf phenotype and drought tolerance by influencing protein expression patterns and ROS scavenging in rice (*Oryza sativa* L.). Agronomy 9:728

Lilley JM, Ludlow MM, McCouch SR, O'toole JC (1996) Locating QTL for osmotic adjustment and dehydration tolerance in rice. J Exp Bot 47:1427–1436

Lister C, Dean C (1993) Recombinant inbred lines formapping RFLP and phenotypic markers in *Arabidopsis thaliana*. Plant J 4:745–750

Liu K, Wang L, Xu Y, Chen N, Ma Q, Li F, Chong K (2007) Overexpression of OsCOIN, a putative cold inducible zinc finger protein, increased tolerance to chilling, salt and drought, and enhanced proline level in rice. Planta 226:1007–1016

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 X, Li M, Liu K, Tang D, Sun M, Li Y, Shen Y, Du G, Cheng Z (2016) *Semi-Rolled Leaf2* modulates rice leaf rolling by regulating abaxial side cell differentiation. J Exp Bot 67:2139–2150

Long A, Zhang J, Yang LT, Ye X, Lai NW, Tan LL et al (2017) Effects of low pH on photosynthesis, related physiological parameters and nutrient profile of *Citrus*. Front Plant Sci 8:185–190

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

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

Lum MS, Hanafi MM, Rafii YM, Akmar ASN (2014) Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. J Anim Plant Sci 24:1487–1493

Ma JF, Furukawa J (2003) Recent progress in the research of external Al detoxification in higher plants: a minireview. J Inorg Biochem 97:46–51

Ma JF, Shen R, Zhao Z, Wissuwa M, Takeuchi Y, Ebitani T, Yano M (2002) Response of rice to Al stress and identification of quantitative trait loci for Al tolerance. Plant Cell Physiol 43 (6):652–659

Ma JF, Chen ZC, Shen RF (2014) Molecular mechanisms of Al tolerance in gramineous plants. Plant Soil 381:1–12

Magalhaes J, Garvin D, Wang Y, Sorrells M, Klein P (2004) Comparative mapping of a major aluminum tolerance gene in sorghum and other species in the Poaceae. Genetics 167:1905–1914

Magalhaes JV, Liu J, Guimara CT, Lana UGP, Wang Y, SchaVert RE, Hoekenga OA, Shaff JE, Klein PE, Carneiro NP, Coelho CM, Trick HN, Kochian LV (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat Genet 39:1156–1161

Mahender A, Swamy BPM, Anandan A, Ali J (2019) Tolerance of iron deficient and -toxic soil conditions in rice. Plants 8:31

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, San Diego, CA

Maruyama K, Todaka D, Mizoi J, Yoshida T, Kidokoro S, Matsukura S et al (2012) Identification of cis-acting promoter elements in cold- and dehydration-induced transcriptional pathways in *Arabidopsis*, rice, and soybean. DNA Res 19:37–49

Maruyama K, Urano K, Yoshiwara K, Morishita Y, Sakurai N, Suzuki H et al (2014) Integrated analysis of the effects of cold and dehydration on rice metabolites, phytohormones, and gene transcripts. Plant Physiol 164:1759–1771

Mather K, Caicedo A, Polato N, Olsen K, McCouch S, Purugganan MD (2007) The extent of linkage disequilibrium in rice (*Oryza sativa* L.). Genet 177:2220–2223

Matsukura S, Mizoi J, Yoshida T, Todaka D, Ito Y, Maruyama K et al (2010) Comprehensive analysis of rice DREB2-type genes that encode transcription factors involved in the expression of abiotic stress-responsive genes. Mol Genet Genom 283:185–196

Matsumoto H (2000) Cell biology of aluminum toxicity and tolerance in highe plants. Int Rev Cytol 200:1–46

Matthus E, Wu LB, Ueda Y, Höller S, Becker M, Frei M (2015) Loci, genes, and mechanisms associated with tolerance to ferrous iron toxicity in rice (*Oryza sativa* L.). Theor Appl Genet 128(10):2085–2098

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryzasativa* L.). DNA Res 9:199–207

McCouch SR, Zhao K, Wright M, Tung C-W, Ebana K, Thomson M, Reynolds A, Wang D, DeClerck G, Ali ML, McClung A, Eizenga G, Bustamante C (2010) Development of genome-wide SNP assays for rice. Breed Sci 60:524–535

McNally KL, Childs KL, Bohnert R, Davidson RM, Zhao K, Ulat VJ, Zeller G, Clark RM, Hoen DR, Bureau TE, Stokowski R, Ballinger DG, Frazer KA, Cox DR, Padhukasahasram B, Bustamante CD, Weigel D, Mackill DJ, Bruskiewich RM, Rätsch G, Buell CR, Leung H, Leach JE (2009) Genome-wide SNP variation reveals relationships among landraces and modern varieties of rice. Proc Natl Aced Sci USA 106:12273–12278

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: integrated software for geneticlinkage map construction and quantitative traitlocus mapping in biparental populations. Crop J 3:269–283

Miftahudin, Scholes GJ, Gustafson JP (2002) AFLP markers tightly linked to the aluminum-tolerance gene *Alt3* in *rye* (*Secale cereal* L.). Theor Appl Genet 104:626–631

Miftahudin, Scoles GJ, Gustafson JP (2004) Development of PCR-based codominant markers flanking the *Alt3* gene in rye. Genome 47:231–238

Miftahudin, Chikmawati T, Ross K, Scoles GJ, Gustafson JP (2005) Targeting the aluminum tolerance gene *Alt3* region in *rye*, using *rice/rye* microcolinearity. Theor Appl Genet 110:906–913

Miftahudin, Nurlaela, Juliarni (2007) Uptake and distribution of aluminum in rootapices of two rice varieties under aluminum stress. HAYATI J Biosci 14(3):110–114

Miftahudin, Putri RE, Chikmawati T (2020) Vegetative morphophysiological responses of four rice cultivars to drought stress. Biodiversitas 21:3727–3734

Miftahudin M, Roslim DI, Fendiyanto MH, Satrio RD, Zulkifli A, Umaiyah EI, Chikmawati T, Sulistyaningsih YC, Suharsono S, Hartana A, Nguyen HT, Gustafson JP (2021) *OsGERLP*: a

novel aluminum tolerance rice gene isolated from a local cultivar in Indonesia. Plant Physiol Biochem 162:86–99
Minella E, Sorrells M (1992) Aluminum tolerance in barley: genetic relationships among genotypes of diverse origin. Crop Sci 32:593–598
Mishra KK, Vikram P, Yadaw RB, Swamy BM, Dixit S, Cruz MTS, Maturan P, Marker S, Kumar A (2013) qDTY12.1: a locus with a consistent effect on grain yield under drought in rice. BMC Genetics 14:12
Mitra GN (2015) Regulation of nutrient uptake by plants: a biochemical and molecular approach. Springer, New Dehli, India
Miyakawa T, Fujita Y, Yamaguchi-Shinozaki K, Tanokura M (2013) Structure and function of abscisic acid receptors. Trends Plant Sci 18:259–266
Miyasaka SC, Buta JG, Howell RK, Foy CD (1991) Mechanisms of aluminum tolerance in snapbean. Root exudation of citric acid. Plant Physiol 96:737–743
Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) AP2/ERF family transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819:86–96
Moncada P, Martinez CP, Borrero J, Châtel M, Gauch H Jr, Guimarães E, Tohme J, McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC2F2 population evaluated in an upland environment. Theor Appl Genet 102:41–52
Morimoto K, Mizoi J, Qin F, Kim JS, Sato H, Osakabe Y et al (2013) Stabilization of *Arabidopsis* DREB2A is required but not sufficient for the induction of target genes under conditions of stress. PLoS ONE 8:e80457
Mossor-Pietraszewska TM (2001) Effect of aluminium on plant growth and metabolism. Acta Biochim Pol 48(3):673–686
Mulyani A, Hikmatullah, Subagyo, H (2004) Characteristics and potential of dryland acid soils in Indonesia. Bogor: Indonesian Center for Soil and Agro-climate Research and Development [in Indonesian]
Muthu V, Abbai R, Nallathambi J, Rahman H, Ramasamy S, Kambale R et al (2020) Pyramiding QTLs controlling tolerance against drought, salinity, and submergence in rice through marker assisted breeding. PLoS ONE 15:e0227421
Muthurajan R, Rahman H, Muthukumar M, Ramanathan V, Selvam J (2018) Drought responsive transcriptome profiling in roots of contrasting rice genotypes. Indian J Plant Physiol 23:393–407
Myers SS, Smith MR, Sarah G, Golden CD, Bapu V, Mueller ND, Dangour AD, Peter H (2017) Climate change and global food systems: potential impacts on food security and undernutrition. Annu Rev Publ Health 38:259–277
Nakashima K, Tran LSP, Van Nguyen D, Fujita M, Maruyama K, Todaka D 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
Nakashima K, Takasaki H, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) NAC transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819:97–103
Nelson GCD, Mensbrugghe V, Ahammad H (2014) Agriculture and climate change in global scenarios: why don't the models agree. Agric Econ 45:85–101
Nguyen VT, Burow MD, Nguyen HT, Le BT, Le TD, Paterson AH (2001) Molecular mapping of genes coffering aluminum tolerance in rice (*Oryza sativa* L.). Theor Appl Genet 102:1002–1010
Nguyen VT, Nguyen BD, Sarkarung S, Martinez C, Paterson AH, Nguyen HT (2002) Mapping of genes controlling aluminum tolerance in rice: comparison of different genetics background. Mol Genet Genom 267:772–780
Nguyen BD, Brar DS, Bui BC, Nguyen TV, Pham LN, Nguyen HT (2003) Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff. into indica rice (*Oryza sativa* L.). Theor Appl Genet 106:583–593
Nijhawan A, Jain M, Tyagi AK, Khurana JP (2008) Genomic survey and gene expression analysis of the basic leucine zipper transcription factor family in rice. Plant Physiol 146:333–350

Ninamango-Cardenas F, Teixeira Guimarães C, Martins P, NettoParentoni S, Carneiro NP, Lopes MA, Moro JR, Paiva E (2003) Mapping QTLs for aluminum tolerance in maize. Euphytica 130:223–232

Ning Y, Jantasuriyarat C, Zhao Q, Zhang H, Chen S, Liu J, Liu L, Tang S, Park CH, Wang X, Liu X, Dai L, Xie Q, Wang GL (2011) The SINA E3 ligase OsDIS1 negatively regulates drought response in rice. Plant Physiol 157:242–255

Ningsih RD, Yasin M, Noor A (2020) Rice productivity on tidal swampland in the agricultural assisitance area program in Barito Kuala Regency South Kalimantan. IOP conference series: Earth Environ Sci 484:012123. https://doi.org/10.1088/1755-1315/484/1/012123

Noor A, Lubis I, Ghulamahdi M, Chozin MA, Anwar K, Wirnas D (2012) Pengaruh konsentrasi Fe dalam larutan hara terhadap gejala keracunan Fe dan pertumbuhan tanaman padi. J Agron Indonesia 40(2):91–98

Noorsyamsi H, Anwarhan H, Soelaiman S, Beachell HM (1984) Rice cultivation in the tidal swamps of Kalimantan. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June 1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 17–28

Nordborg M, Borevitz JO, Bergelson J, Berry CC, Chory J, Hagenblad J, Kreitman M, Maloof JN, Noyes T, Oefner PJ, Stahl EA, Weigel D (2002) The extent of linkage disequilibrium in *Arabidopsis thaliana*. Nat Genet 30:190–193

Nozoe T, Agbisit R, Fukuta Y, Rodriguez R, Yanagihara S (2008) Characteristics of iron tolerant rice lines developed at IRRI under field conditions. Jarq-Jpn Agric Res Q42(3):187–192

Nozoye T, Nagasaka S, Kobayashi T, Takahashi M, Sato Y, Uozume N, Nakanishi H, Nishizawa NK (2011) Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. J Biol Chem 286(7):5446–5454

Nugraha Y, Rumanti IA (2017) Breeding for rice variety tolerant to iron toxicity. Iptek Tan Pangan 12(1):9–24

Nugraha Y, Rumanti IA, Guswara A, Ardie SW, Ghulammahdi M, Suwarno, Aswidinnoor H (2016) Response of selected rice varieties under excess iron condition. J Pen Pert Tan Pangan 35(3):181–190

Nugroho K, Alkasuma, Paidi, Wahdini W, Abdulrachman, Suhardjo H, Widjaja-Adhi IPG (1991) Final Report: Determination of potential areas for tidal lands, swamps, and beaches at a scale of 1: 500,000 (Laporan Teknik No. 1/PSRP/1991). Bogor: Indonesian Center for Soil and Agro-climate Research and Development [in Indonesian]

Nuruzzaman M, Sharoni AM, 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, Song SI, Kim YS, Jang H, Kim SY, Kim M et al (2005) *Arabidopsis* CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol 138:341–351

Oh SJ, Kwon CW, Choi DW, Song SI, Kim JK (2007) Expression of barley HvCBF4 enhances tolerance to abiotic stress in transgenic rice. Plant Biotechnol J 5:646–656

Oh SJ, Kim YS, Kwon CW, Park HK, Jeong JS, Kim JK (2009) Overexpression of the transcription factor AP37 in rice improves grain yield under drought conditions. Plant Physiol 150:1368–1379

Oladosu Y, Rafii MY, Samuel C, Fatai A, Magaji U, Kareem I, Kamarudin ZS, Muhammad I, Kolapo K (2019) Drought resistance in rice from conventional to molecular breeding. Intl J Mol Sci 20:3519

Olsen K, Caicedo A, Polato N, McClung A, McCouch S, Purugganan MD (2006) Selection under domestication: evidence for a sweep in the rice Waxy genomic region. Genetics 173:975

Onaga G, Edema R, Asea G (2013) Tolerance of rice germplasm to iron toxicity stress and the relationship between tolerance, Fe^{2+}, P and K content in the leaves and roots. Arch Agron Soil Sci 59(2):213–229

Onaga G, Dramé KN, Ismail AM (2016) Understanding the regulation of iron nutrition: can it contribute to improving iron toxicity tolerance in rice? Func Plant Biol 43(8):709–726

Osakabe Y, Osakabe K, Shinozaki K, Tran LP (2014) Response of plants to water stress. Plant Physiol 5:1–8

Ouedraogo S, Ouedraogo M (2003) Evaluation économique de l'impact de la recherche et de la vulgarisation sur le riz au Burkina Faso. Mini-report, Institut de l'Environnement et des Recherches Agricoles (INERA), Ouagadougou

Ouyang Y, Zeng F, Zhuang J, Yu S, Zhu L, Jin Q, Zhang G (2007) Genetic analysis of genotype × iron nutrition interaction on coleoptile elongation rate in rice (*Oryza sativa* L.). Euphytica 156:311–318

Ozga JA, Kaur H, Savada RP, Reinecke DM (2016) Hormonal regulation of reproductive growth under normal and heat-stress conditions in legume and other model crop species. J Exp Bot 68:1885–1894

Palanog AD, Swamy BM, Shamsudin NAA, Dixit S, Hernandez JE, Boromeo TH, Cruz PCS, Kumar A (2014) Grain yield QTLs with consistent-effect under reproductive-stage drought stress in rice. Field Crop Res 161:46–54

Phung T, Jung HI, Park J, Kim JG, Back K, Jung S (2011) Porphyrin biosynthesis control under water stress: sustained porphyrin status correlates with drought tolerance in transgenic rice. Plant Physiol 157:1746–1764

Pineros M, Shaff J, Manslank H, Carvalho Alves V, Kochian L (2005) Aluminum resistance in maize cannot be solely explained by root organic acid exudation, a comparative physiological study. Plant Physiol 137:220–231

Praba ML, Cairns JE, Babu RC, Lafitte HR (2009) Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. J Agron Crop Sci 195:30–46

Prince SJ, Beena R, Gomez SM, Senthivel S, Babu RC (2015) Mapping consistent rice (*Oryza sativa* L.) yield QTLs under drought stress in target rainfed environments. Rice 8:25

Pulver E, Jaramillo S, Moreira S, Zorilla G (2010) Catching the rains. Rice Today 9(3):14–16

Qin F, Sakuma Y, Tran LSP, Maruyama K, Kidokoro S, Fujita Y et al (2008) *Arabidopsis* DREB2A-interacting proteins function as RING E3 ligases and negatively regulate plant drought stress-responsive gene expression. Plant Cell 20:1693–1707

Qu P, Shi J, Chen T, Chen K, Shen C, Wang J, Zhao X, Ye G, Xu J, Zhang L (2020) Construction and integration of genetic linkage maps from three multi parent advanced generation intercross populations in rice. Rice 13:13

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

Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010) ABA perception and signalling. Trends Plant Sci 15:395–401

Rakshit S, Rakshit A, Matsumura H, Takahashi Y, Hasegawa Y, Ito A, Ishii T, Miyashita NT, Terauchi R (2007) Large-scale DNA polymorphism study of *Oryza sativa* and *O. rufipogon* reveals the origin and divergence of Asian rice. Theor Appl Genet 114:731–743

Ramírez LM, Claassen N, Ubiera AA, Werner H, Moawad AM (2002) Effect of phosphorus, potassium and zinc fertilizers on iron toxicity in wetland rice (*Oryza sativa* L.). Plant Soil 239:197–206

Ratnasari T, Tjahjoleksono A, Miftahudin (2016) Transgene insertion stability and aluminum tolerance candidate gene expression in T3 generation of transgenic tobacco. Int J Agric Biol 18:607–614

Redillas MCFR, Jeong JS, Kim YS, Jung H, Bang SW, Choi YD et al (2012) The overexpression of OsNAC9 alters the root architecture of rice plants enhancing drought resistance and grain yield under field conditions. Plant Biotechnol J 10:792–805

Reich D, Cargill M, Bolk S, Ireland J, Sabeti P, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES (2001) Linkage disequilibrium in the human genome. Nature 411:199–204

Remington D, Thornsberry J, Matsuoka Y, Wilson L, Whitt S, Doebley J, Kresovich S, Goodman MM, Buckler ES (2001) Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proc Natl Acad Sci USA 98:11470–11479

Reyes-Díaz M, Inostroza-Blancheteau C, Rengel Z (2015) Physiological and molecular regulation of aluminum resistance in woody plant species. In: Panda SK, Baluška F (eds) Aluminum stress adaptation in plants. Springer, Cham, Switzerland, pp 187–202

Ricachenevsky FK, Sperotto RA (2014) There and back again, or always there? The evolution of rice combined strategy for Fe uptake. Front Plant Sci 5:189

Ricachenevsky FK, Sperotto RA, Menguer PK, Fett JP (2010) Identification of Fe-excess-induced genes in rice shoots reveals a WRKY transcription factor responsive to Fe, drought and senescence. Mol Biol Rep 37(8):3735–3745

Ritung S, Suryani E, Subardja D, Sukarman Nugroho K, Suparto Hikmatullah, Mulyani A, Tafakresnanto C, Sulaeman Y, Subandiono RE, Wahyunto P, Prasodjo N, Suryana U, Hidayat H, Priyono A, Supriatna W (2015) Indonesian agricultural land resources: size, distribution, and potential availability. Indonesian Agency for Agricultural Research and Development Press, Jakarta (in bahasa)

Roca-Paixão JF, Gillet FX, Ribeiro TP, Bournaud C, Lourenco-Tessutti T, Noriega DD, Melo B, Almeida-Engler J, Grossi-de-Sa MF (2019) Improved drought stress tolerance in *Arabidopsis* by CRISPR/dCas9 fusion with a histone acetyl transferase. Sci Rep 9:8080

Rohila JS, Jain RK, Wu R (2002) Genetic improvement of basmati rice for salt and drought tolerance by regulated expression of a barley HVA1 cDNA. Plant Sci 163:525–532

Roslim DW (2011) Isolasi dan Karak terisasi Gen Toleran aluminium dari Tanaman Padi. IPB Press, Bogor

Ruengphayak S, Ruanjaichon V, Saensuk C, Phromphan S, Tragoonrung T, Kongkachuichai R, Vanavichit A (2015) Forward screening for seedling tolerance to Fe toxicity reveals a polymorphic mutation in ferric chelate reductase in rice. Rice 8:3

Ryan PR, Delhaize E, Randall PJ (1995) Characterization of Al-stimulated effluxof malate from the pices of Al-tolerant wheat roots. Planta 196:103–110

Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E (2011) The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. J Exp Bot 62(1):9–20

Sahebi M, Hanafi MM, Rafii MY, Mahmud TMM, Azizi P, Osman M, Abiri R, Taheri S, Kalhori N, Shabanimofrad M, Miah G, Atabaki N (2018) Improvement of drought tolerance in rice (*Oryza sativa* L.): genetics, genomic tools, and the WRKY gene family. Bio Med Res Intl 2018:2314–6133

Sahrawat KL, Singh BN (1998) Seasonal differences in iron toxicity tolerance of lowland rice cultivars. Intl Rice Res Notes 23(1):18–19

Saijo Y, Hata S, Kyozuka J, Shimamoto K, Izui K (2000) Over-expression of a single Ca2+-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. Plant J 23:319–327

Saikumar S, Gouda PK, Saiharini A, Varma CMK, Vineesh O, Padmavathi G, Shenoy VV (2014) Major QTL for enhancing rice grain yield under lowland reproductive drought stress identified using an *O. sativa*/*O. glaberrima* introgression line. Field Crop Res 163:119–131

Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K (2006a) Functional analysis of an *Arabidopsis* transcription factor, DREB2a, involved in drought-responsive gene expression. Plant Cell 18:1292–1309

Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K (2006b) 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

Sandhu N, Singh A, Dixit S, Cruz MTS, Maturan PC, Jain RK, Kumar A (2014) Identification and mapping of stable QTL with main and epistasis effect on rice grain yield under upland drought stress. BMC Genet 15:63

Sandhu M, Sureshkumar V, Prakash C, Dixit R, Solanke AU, Sharma TR, Mohapatra T, Mithra SVA (2017) RiceMetaSys for salt and drought stress responsive genes in rice: a web interface for crop improvement. BMC Bioinformatics 18:432

Sasaki T, Yamamoto Y, Ezaki E, Katsuhara M, Ryan PR, Delhaize E, Matsumoto H (2004) A gene encoding an aluminum-activated malate transporter segregates with aluminum tolerance in wheat. Plant J 37:645–653

Sasaki T, Ryan P, Delhaize E, Hebb D, Ogihara Y (2006) Sequence upstream of the wheat (*Triticum aestivum* L.) ALMT1 gene and its relationship to aluminum resistance. Plant Cell Physiol 47:1339–1343

Satrio RD, Fendiyanto MH, Supena EDJ, Suharsono S, Miftahudin M (2019) Identification of drought-responsive regulatory genes by hierarchical selection of expressed sequence tags and their expression under drought stress in rice. Intl J Agric Biol 22:1524–1532

Satrio RD, Fendiyanto MH, Supena EDJ, Suharsono S, Miftahudin M (2020) Genome-wide *SNP* discovery, linkage mapping, and analysis of QTL for morpho-physiological traits in rice during vegetative stage under drought stress condition. Euphytica (in press)

Schneider K (2005) Mapping populations and principles of genetic mapping in the handbook of plant genome mapping, genetic and physical mapping. WILEY-VCH Verlag, Weinheim

Sellamuthu R, Liu GF, Ranganathan CB, Serraj R (2011) Genetic analysis and validation of quantitative trait loci associated with reproductive-growth traits and grain yield under drought stress in a doubled haploid line population of rice (*Oryza sativa* L.). Field Crops Res 124:46–58

Seo JS, Joo J, Kim MJ, Kim YK, Nahm BH, Song SI et al (2011) OsbHLH148, a basic helix-loop-helix protein, interacts with OsJAZ proteins in a jasmonate signaling pathway leading to drought tolerance in rice. Plant J 65:907–921

Serraj R, McNally KL, Slamet-Loedin I, Kohli A, Haefele SM, Atlin G, Kumar A (2011) Drought resistance improvement in rice: an integrated genetic and resource management strategy. Plant Production Sci 1–14

Shen H, Liu C, Zhang Y, Meng X, Zhou X, Chu C et al (2012) OsWRKY30 is activated by MAP kinases to confer drought tolerance in rice. Plant Mol Biol 80:241–253

Shimizu A (2009) QTL analysis of genetic tolerance to iron toxicity in rice (*Oryza sativa* L.) by quantification of bronzing score. J New Seeds 10(3):171–179

Shimizu A, Guerta CQ, Gregorio GB, Ikehashi H (2005) Improved mass screening of tolerance to iron toxicity in rice by lowering temperature of culture solution. J Plant Nut 28(9):1481–1493

Sikirou M, Saito K, Achigan-Dako EG, Dramé KN, Ahanchédé A, Venuprasad R (2015) Genetic improvement of iron toxicity tolerance in rice—progress, challenges and prospects in West Africa. Plant Prod Sci 18(4):423–434

Sikirou M, Saito K, Dramé KN, Saidou A, Dieng I, Ahanchédé A, Venuprasad R (2016) Soil-based screening for iron toxicity tolerance in rice using pots. Plant Prod Sci 19(4):489–496

Simpson SP (1989) Detection of linkage between quantitative trait loci and restriction fragment length polymorphisms using inbred lines. Theor Appl Genet 77:815–819

Sinclair TR (2011) Challenges in breeding for yield increase for drought. Trends Plant Sci 16 (6):289–293

Sinha TS, Bandyopadhyay AK (1984) Rice in coastal saline land of West Bengal, India. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June 1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 115–118

Siska DM, Hamim, Miftahudin (2017) Overexpression of *B11* gene in transgenic rice increased tolerance to aluminum stress. HAYATI J Biosci 24:96–104

Sivamani E, Bahieldin A, Wraith JM, Al-Niemi T, Dyer WE, Ho THD, Qu R (2000) Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley *HVA1* gene. Plant Sci 155:1–9

Soepardi HG (2001) Land resource-based agribusiness farming strategy. Bogor: Indonesian Center for Soil and Agro-climate Research and Development [in Indonesian]

Song SY, Chen Y, Chen J, Dai XY, Zhang WH (2011) Physiological mechanisms underlying OsNAC5-dependent tolerance of rice plants to abiotic stress. Planta 234:331–345

Srividya A, Vemireddy LR, Ramanarao PV, Sridhar S, Jayaprada M, Anuradha G, Srilakshmi B, Reddy HK, Hariprasad AS, Siddiq EA (2011) Molecular mapping of QTLs for drought related traits at seedling stage under PEG induced stress conditions in rice. Amer J Plant Sci 2:190

Stein RJ, Lopes SIG, Fett JP (2014) Iron toxicity in field-cultivated rice: contrasting tolerance mechanisms in distinct cultivars. Theor Exp Plant Physiol 26(2):135–146

Subagyo H, Widjaja-Adhi IPG (1998) Opportunities and constraints for swampland development for agricultural development in Indonesia. Bogor: Indonesian Center for Soil and Agro-climate Research and Development [in Indonesian]

Subagyo H, Suharta N, Siswanto AB (2000) Agricultural lands in Indonesia. Bogor: Indonesian Center for Soil and Agro-climate Research and Development [in Indonesian]

Suhartini T, Suwarno, Safaruddin (1996) Genetic parameters of iron toxicity tolerance in rice using diallel analysis. Zuriat 7(1):33–40 [in Indonesian]

Su J, Wu R (2004) Stress-inducible synthesis of proline in transgenic rice confers faster growth under stress conditions than that with constitutive synthesis. Plant Sci 166:941–948

Suhartini T, Makarim AK (2009) Teknik seleksi genotipe padi toleran keracunan besi. J Penelit Pertan Tanam Pangan 28:125–130

Takahashi F, Kuromori T, Urano K, Yamaguchi-Shinozaki K, Shinozaki K (2020) Drought stress responses and resistance in plants: from cellular responses to long-distance intercellular communication. Front Plant Sci 11:1–14

Takasaki H, Maruyama K, Kidokoro S, Ito Y, Fujita Y, Shinozaki K et al (2010) The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice. Mol Genet Genom 284:173–183

Tang Y, Sorrells ME, Kochian LV, Garvin DF (2000) Identification of RFLP markers linked to the barley aluminum tolerance gene *Alp*. Crop Sci 40:778–782

Tang N, Zhang H, Li X, Xiao J, Xiong L (2012) Constitutive activation of transcription factor OsbZIP46 improves drought tolerance in rice. Plant Physiol 158:1755–1768

Tenaillon M, Sawkins M, Long A, Gaut R, Doebley J, Gaut BS (2001) Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.). Proc Natl Acad Sci USA 98:9155–9161

Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Martinez CP, McCouch SR (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor Appl Genet 107:479–493

Timmer P (2010) Food security in Asia and the changing role of rice. The Asia Foundation Occasional Paper No. 4

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 6:1–20

Tripathy JN, Zhang J, Robin S, Nguyen TT, Nguyen HT (2000) QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. Theor Appl Genet 100:1197–1202

Tsutsui T, Yamaji N, Ma JF (2011) Identification of a *cis*-acting element ofART1, a C2H2-type zinc-finger transcription factor for aluminum tolerancein rice. Plant Physiol 156:925–931

Tung C-W, Zhao K, Wright M, Ali M, Jung J, Kimball J, Tyagi W, Thomson MJ, McNally K, Leung H, Kim H, Ahn SN, Reynolds A, Scheffler B, Eizenga G, McClung A, Bustamante C, McCouch SR (2010) Development of a research platform for dissecting phenotype–genotype associations in rice (*Oryza* spp.). Rice 1:1–13

Turhadi T, Hamim H, Ghulamahdi M, Miftahudin M (2018) Morpho-physiological responses of rice genotypes and its clustering under hydroponic iron toxicity conditions. Asian J Agri Biol 6 (4):495–505

Turhadi T, Hamim H, Ghulamahdi M, Miftahudin M (2019) Iron toxicity-induced physiological and metabolite profile variations among tolerant and sensitive rice varieties. Plant Signal Behav 14(12):1682829

Turhadi T, Hamim H, Ghulamahdi M, Miftahudin M (2020) Morpho-physiological and anatomical character changes of rice under waterlogged and water-saturated acidic and high Fe content soil. Sains Malaysiana 49(10):2411–2424

Umezawa T, Nakashima K, Miyakawa T, Kuromori T, Tanokura M, Shinozaki K et al (2010) Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. Plant Cell Physiol 51:1821–1839

Usman MG, Rafii MY, Martini MY, Yusuff OA, Ismail MR, Miah G (2017) Molecular analysis of Hsp70 mechanisms in plants and their function in response to stress. Biotechnol Genet Eng Rev 33:26–39

Utami DW, Hanarida I (2014) Evaluasi lapang dan identifikasi molekuler plasma nutfah padi terhadap keracunan Fe. Agro Biogen 10(1):9–17

Varshney RK, Pandey MK, Chitikineni A (2018) Plant genetics and molecular biology: an introduction. In: Varshney RK, Pandey MK, Chitikineni A (eds) Plant genetics and molecular biology. Advances in biochemical engineering/biotechnology, vol 164. Springer, pp 1–9

Venuprasad R, Shashidhar HE, Hittalmani S, Hemamalini GS (2002) Tagging quantitative trait loci associated with grain yield and root morphological traits in rice (*Oryza sativa* L.) under contrasting moisture regimes. Euphytica 128:293–300

Verma SK, Saxena RR, Saxena RR, Xalxo MS, Verulkar SB (2014) QTL for grain yield under water stress and non-stress conditions over years in rice (*Oryza sativa* L.). Aus J Crop Sci 8:916–926

Vikram P, Swamy BM, Dixit S, Cruz MT, Ahmed HU, Singh AK, Kumar A (2011) qDTY1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. BMC Genetics 12:89

Vinod KK, Krishnan SG, Thribhuvan R, Singh AK (2019) Genetics of drought tolerance, mapping QTLs, candidate genes and their genetics of drought tolerance, mapping QTLs, candidate genes and their utilization in rice improvement. In: Rajpal VR, Sehgal D, Kumar A, Raina SN (eds) Genomics assisted breeding of crops for abiotic stress tolerance, vol II, sustainable development and biodiversity 21. Springer, pp 145–186

Virmani SS (1977) Varietal tolerance of rice to iron toxicity in Liberia. Intl Rice Res Newsl Int Rice Res Inst 2:4–5

von Uexkuell HR, Mutert E (1995) Global extent, development and economic impact of acid soils. Plant Soil 171:1–15

Wahyuningtyas W, Miftahudin Widyastuti U, Tjahjoleksono A (2016) Construction of RNA interference vector to silence aluminumtolerance gene candidate in rice cv HawaraBunar. HAYATI J Biosci 23:79–84

Wailes EJ, Chavez EC (2012) World rice outlook-international rice baseline with deterministic and stochastic projections 2012–2021. Department of Agricultural Economics and Agribusiness, University of Arkansas, Fayetteville

Wallace JG, Rodger-Melnick E, Buckler ES (2018) On the road to breeding 4.0: unraveling the good, the bad, and the boring of crop quantitative genomics. Annu Rev Genet 52:26.1–26.24

Wan JL, Zhai HQ, Wan JM, Ikehashi H (2003) Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice, *Oryza sativa* L. Euphytica 131:201–206

Wan JL, Zhai HQ, Wan JM, Yasui H, Yoshimura A (2004) Detection and analysis of QTLs associated with resistance to ferrous iron toxicity in rice (*Oryza sativa* L.), using recombinant inbred lines. Zuo Wu Xue Bao 30:329–333

Wan JL, Zhai HQ, Wan JM (2005) Mapping of QTLs for ferrous iron toxicity tolerance in rice (*Oryza sativa* L.). Yi Chuan Xue Bao 32(11):1156–1166

Wan L, Zhang J, Zhang H, Zhang Z, Quan R, Zhou S et al (2011) Transcriptional activation of OsDERF1 in OsERF3 and OsAP2-39 negatively modulates ethylene synthesis and drought tolerance in rice. PLoS ONE 6:e25216

Wang Q, Tang J, Han B (2020a) Advances in genome-wide association studies of complex traits in rice. Theor Appl Genet 133:1415–1425

Wang F, Itai RN, Nozoye T, Kobayashi T, Nishizawa NK, Nakanishi H (2020b) The bHLH protein OsIRO3 is critical for plant survival and iron (Fe) homeostasis in rice (*Oryza sativa* L.) under Fe-deficient conditions. Soil Sci Plant Nutr. https://doi.org/10.1080/00380768.2020.1783966

WARDA (1998) Annual Report 1997: West Africa Rice Development Association, Bouaké
Watanabe T, Osaki M (2001) Influence of aluminum and posphorus on groTLhand xylem sap composition in *Melastomamala batricum* L. Plant Soil 237:63–70
Watson GA (1984) Utility of rice cropping strategies in Semuda Kecil village, Central Kalimantan, Indonesia. In: International Rice Research Institute (ed) The workshop on research priorities in tidal swamp rice; 22–25 June 1981; Banjarmasin, South Kalimantan, Indonesia. International Rice Research Institute, Manila, pp 49–68
Wan JL, Zhai HQ, Wan JM, Yasui H, Yoshimura A (2003a) Mapping QTL for traits associated with resistance to ferrous iron toxicity in rice (*Oryza sativa* L.), using japonica chromosome segment substitution lines. Yi Chuan Xue Bao 30(10):893–898
Wan JL, Zhai HQ, Wan JM, Ikehashi H (2003b) Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice, *Oryza sativa* L. Euphytica 131:201–206
Weiner JJ, Peterson FC, Volkman BF, Cutler SR (2010) Structural and functional insights into core ABA signaling. Curr Opin Plant Biol 13:495–502
Widjaja-Adhi IPG, Suriadikarta DA, Sutriadi MT, Subiksa IGM, Suastika IW (2000) Management of swamp utilization and development. Bogor: Indonesian Center for Soil and Agro-climate Research and Development [in Indonesian]
Wijayanto A (2013) Aplikasi Marka Molekulerdalam Seleksi Populasi Silang Balik IR64/Hawara Bunaruntuk Mendapatkan Galur Padi Toleran aluminium. IPB Press, Bogor
Wood S, Sebastian K, Cherr SJS (2000) Pilot Analysis of Global Ecosystems Agroecosystems. United States of America: World Resources Institute
Wu P, Hu B, Liao CY, Zhu JM, Wu YR, Senadhira D, Paterson AH (1998) Characterization of tissue tolerance to iron by molecular markers in different lines of rice. Plant Soil 203:217–226
Wu P, Liao CY, Hu B, Yi KK, Jin WZ, Ni JJ, He C (2000) QTLs and epistasis foraluminum tolerance in rice (*Oryza sativa* L.) at different seedling stages. Theor Appl Genet 100:1295–1303
Wu LB, Shhadi MY, Gregorio G, Matthus E, Becker M, Frei M (2014) Genetic and physiological analysis of tolerance to acute iron toxicity in rice. Rice 7(1):1–8
Wu LB, Ueda Y, Lai SK, Frei M (2016) Shoot tolerance mechanisms to iron toxicity in rice (*Oryza sativa* L.). Plant Cell Environ 40(4):570–584
Xia JX, Yamaji N, Kasai T, Ma JF (2010) Plasma membrane localized transporterfor aluminum in rice. Proc Natl Acad Sci USA 107:18381–18385
Xia J, Yamaji N, Ma JF (2011) Further characterization of an aluminum influx transporter in rice. Plant Signal Behav 6:160–173
Xia JX, Yamaji N, Ma JF (2013) A plasma membrane-localized smallpeptide is involved in Al tolerance in rice. Plant J76:345–355
Xia J, Yamaji N, Che J, Shen RF, Ma JF (2014) Differential expression of *Nrat1* is responsible for Al-tolerance QTL on chromosome 2 in rice. J Exp Bot 65(15):4297–4304
Xiang Y, Huang Y, Xiong L (2007) Characterization of stress-responsive CIPK genes in rice for stress tolerance improvement. Plant Physiol 144:1416–1428
Xiang Y, Tang N, Du H, Ye H, Xiong L (2008) Characterization of OsbZIP23 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
Xiao B, Huang Y, Tang N, Xiong L (2007) Over-expression of a LEA gene in rice improves drought resistance under the field conditions. Theor Appl Genet 115:35–46
Xiao BZ, Chen X, Xiang CB, Tang N, Zhang QF, Xiong LZ (2009) Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. Mol Plant 2:73–83
Xu D, Duan X, Wang B, Hong B, Ho THD, Wu R (1996) Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 110:249–257

Xu JL, Lafitte HR, Gao YM, Fu BY, Torres R, Li ZK (2005) QTLs for drought escape and tolerance identified in a set of random introgression lines of rice. Theor Appl Genet 111:1642–1650

Xu DQ, Huang J, Guo SQ, Yang X, Bao YM, Tang HJ et al (2008) Over-expression of a TFIIIA-type zinc finger protein gene ZFP252 enhances drought and salt tolerance in rice (*Oryza sativa* L.). FEBS Lett 582:1037–1043

Xue Y, Wan JM, Jiang L, Liu LL, Su N, Zhai HQ, Ma JF (2006) QTL analysis of aluminum resistance in rice (*Oryza sativa* L.). Plant Soil 287:375–383

Xue Y, Jiang L, Su N, Wang JK, Deng P, Ma JF, Zhai HQ, Wan JM (2007) The genetic basic and fine-mapping of a stable quantitative-trait loci for aluminum tolerance in rice. Planta 22 (7):255–262

Yadira OC, Reyes JL, Alejandra AC (2011) Late embryogenesis abundant proteins. Plant Signal Behav 6:586–589

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

Yamaji N, Huang CF, Nagao S, Yano M, Sato Y, Nagamura Y, Ma JF (2009) A zing finger transcription factor *ART1* regulates multiple genes implicated in aluminum tolerance in rice. Plant Cell 21:3339–3349

Yamauchi M (1989) Rice bronzing in Nigeria caused by nutrient imbalances and its control by potassium sulfate application. Plant Soil 117:275–286

Yang A, Dai X, Zhang WH (2012) A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice. J Exp Bot 63:2541–2556

Yang LT, Qi YP, Jiang HX, Chen LS (2013) Roles of organic acid anion secretion in aluminium tolerance of higher plants. Biomed Res Int 13:173682

Yin K, Gao C, Qiu JL (2017) Progress and prospects in plant genome editing. Nat Plants 3:1–6

Yokosho K, Yamaji N, Ma JF (2011) An Al-inducible MATE gene is involved inexternal detoxification of Al in rice. Plant Cell 68:1061–1069

Yokosho K, Yamaji N, Fujii-Kashino M, Ma JF (2016) Retrotransposon-mediated aluminum tolerance through enhanced expression of the citrate transporter OsFRDL4. Plant Physiol 172:2327–2336

Yoshida S (1981) Fundamentals of rice crop science. International Rice Research Institute, Los Banos, Manila, Philippines

Yoshida S, Forno DA, Cock JH, Gomez KA (1976) Laboratory manual for physiological studies of rice. The International Rice Research Institute, Manila, Philippines

You J, Hu H, Xiong L (2012) An ornithine δ-aminotransferase gene OsOAT confers drought and oxidative stress tolerance in rice. Plant Sci 197:59–69

You J, Zong W, Li X, Ning J, Hu H, Li X, Xiong L (2013) The SNAC1-targeted gene OsSRO1c modulates stomata closure and oxidative stress tolerance by regulating hydrogen peroxide in rice. J Exp Bot 64:569–583

Yu J, Pressoir G, Briggs W, Bi I, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2005) A unified mixed model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208

Yu CS, Chen YC, Lu CH, Hwang JK (2006) Prediction of protein subcellular localization. Proteins Struct Funct Bioinf 64:643–651

Yue B, Xue W, Luo L, Xing Y (2008) Identification of quantitative trait loci for four morphologic traits under water stress in rice (*Oryza sativa* L.). J Gene Genom 35:569–575

Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD, Sarkarung S (2001) Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. Theor Appl Genet 103:19–29

Zhang H, Liu W, Wan L, Li F, Dai L, Li D et al (2010a) Functional analyses of ethylene response factor JERF3 with the aim of improving tolerance to drought and osmotic stress in transgenic rice. Transgen Res 19:809–818

Zhang Z, Li F, Li D, Zhang H, Huang R (2010b) Expression of ethylene response factor JERF1 in rice improves tolerance to drought. Planta 232:765–774

Zhang L, Xiao S, Li W, Feng W, Li J, Wu Z, Shao M (2011) Overexpression of a Harpin-encoding gene hrf1 in rice enhances drought tolerance. J Exp Bot 62:4229–4238

Zhang H, Zhang J, Quan R, Pan X, Wan L, Huang R (2013) EAR motif mutation of rice OsERF3 alters the regulation of ethylene biosynthesis and drought tolerance. Planta 237:1443–1451

Zhang P, Zhong K, Tong H, Shahid MQ, Li J (2016) Association mapping for aluminum tolerance in a core collection of rice landraces. Front Plant Sci 7(1415):1–11

Zhang J, Chen K, Pang Y, Naveed SA, Zhao X, Wang X, Wang Y, Dingkuhn M, Pasuquin J, Li Z (2017) QTL mapping and candidate gene analysis of ferrous iron and zinc toxicity tolerance at seedling stage in rice by genome-wide association study. BMC Genom 18:828

Zhao K, Aranzana M, Kim S, Lister C, Shindo C, Tang C, Toomajian C, Zheng H, Dean C, Marjoram P, Nordborg M (2007) An *Arabidopsis* example of association mapping in structured samples. PLoS Genet 3:e4

Zhao K, Tung CW, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Bustamante CD, McCouch SR (2011) Genome-wide association mapping reveals rich genetic architecture of complex traits in *Oryza sativa*. Nat Commun 2:1–10

Zheng SJ (2010) Iron homeostasis and iron acquisition in plants: maintenance, functions and consequences. Ann Bot 105(6):799–800

Zheng SJ, Yang JL, He YF, Yu XH, Zhang L, You JF, Shen RF, Matsumoto H (2005) Immobilization of aluminum with phosphorus in roots is associated with high Al resistance in buck wheat. Plant Physiol 138:297–303

Zheng B, Yang L, Mao C, Huang Y, Wu P (2008) Comparison of QTLs for rice seedling morphology under different water supply conditions. J Genet Genom 35:473–484

Zhou S, Zhu M, Wang F, Huang J, Wang G (2013) Mapping of QTLs for yield and its componentsin a rice recombinant inbred line population. Pak J Bot 45:183–189

Zhu B, Su J, Chang M, Verma DPS, Fan YL, Wu R (1998) Overexpression of a Δ1-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water-and salt-stress in transgenic rice. Plant Sci 139:41–48

Zong W, Tang N, Yang J, Peng L, Ma S, Xu Y, Li G, Xiong L (2016) Feedback regulation of ABA signalling and biosynthesis by a bZIP transcription factor targets drought-resistance-related genes. Plant Physiol 171(4):2810–2825

Zou GH, Mei HW, Liu HY, Liu GL, Hu SP, Yu XQ, Li MS, Wu JH, Luo LJ (2005) Grain yield responses to moisture regimes in a rice population: association among traits and genetic markers. Theor Appl Genet 112:106–113

Chapter 2
Advances in Breeding for Abiotic Stress Tolerance in Wheat

Suchismita Mondal, Ahmed Sallam, Deepmala Sehgal, Sivakumar Sukumaran, Md Farhad, J. Navaneetha Krishnan, Uttam Kumar, and Akshaya Biswal

Abstract Wheat is a key economically important cereal crop that is consumed globally. While the grain yield increase is steady at around 1%, it is not enough to meet the growing global demands of the next decades. One the major factor that affects wheat production is the uncertainty in climatic patterns. High temperature, drought, frost, and salinity are some of the abiotic stresses known to affect wheat production significantly. Developing wheat varieties with stable and high grain yield is the crucial for sustainable wheat production. Though, diversity for tolerance to abiotic stress exists within the wheat gene pools and elite germplasms, there is a need to rapidly introgress and breed for stress adapted lines. Optimization of the breeding process, through use of effective screening technologies, faster generation advance, and recycling of parents could impact the varietal development process significantly. The advances in genomic technologies, such as better and cheaper molecular markers and improved prediction models for genomic selection could further contribute to breeding for stress tolerant germplasm. Opportunities exists to increase the grain yield trends under abiotic stresses, which need to be effectively and efficiently utilized.

Keywords Wheat · Heat tolerance · Genomic selection · Genomics · Breeding

S. Mondal (✉) · D. Sehgal · S. Sukumaran · U. Kumar · A. Biswal
Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), 06600 Mexico City, Mexico
e-mail: S.mondal@cgiar.org

A. Sallam
Department of Genetics, Faculty of Agriculture, Assiut University, Asyut 71526, Egypt

M. Farhad · J. Navaneetha Krishnan
TERI School of Advanced Studies, New Delhi, India

M. Farhad
Bangladesh Wheat and Maize Research Institute, Dinajpur, Bangladesh

J. Navaneetha Krishnan
Punjab Agricultural University, Ludhiana, Punjab, India

U. Kumar
Borlaug Institute for South Asia, Ludhiana, Punjab, India

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2.1 Introduction

Wheat (*Triticum aestivum* L.) is a staple cereal crop grown widely and is also economically important in terms of acreage and commerce. It is cultivated in an array of latitudes and altitudes, though commonly cultivated between 30 °N to 60 ° N and 27 °S to 40 °S latitudes, and up to 3000 m above the sea level (Deng et al. 2005). It is also adapted to a broad range of temperature and moisture conditions, from temperate to tropical, with annual precipitation of 250–2000 mm (Deng et al. 2005). Wheat is consumed globally, and the increasing demand drives the production and prices which are ultimately linked to food security. Studies have shown that one of the most important factors that affects the global wheat market, is the extremes in climatic patterns (Enghiad et al. 2017). The uncertainty of weather patterns, especially temperature and rainfall can affect its production significantly. Globally, drought events due to uneven rainfall can result in grain yield losses from 21 to 40% (Daryanto et al. 2016), while high temperatures during grain filling can cause wheat yield losses of 6 to 10% per degree Celsius rise in temperature (Zhao et al. 2017). These reductions in wheat yields are highly heterogenous across geographical areas and depends on the varieties been grown, agronomic practices followed and soil conditions. Stresses are often seen in combination, which further exacerbates the effect on crop yield. In addition to drought and temperature stress, abiotic stresses such as salinity, heavy metal toxicity and frost also affect the grain yields in wheat growing regions.

With the world population increasing at the rate of 1.14% (ONU 2019), a productivity increase of 1.8% per year is predicted by Ray et al. (2013) to fulfill the rising demands by 2050. Though wheat production has increased substantially since the Green Revolution, the annual genetic gains are estimated to be in a range 0.5–1%, in different environmental conditions, which is clearly not enough to meet the growing demands (Sharma et al. 2012; Crespo-Herrera et al. 2017, 2018; Mondal et al. 2020). Given the challenges of abiotic stress, breeders need to accelerate wheat production through the development of stress tolerant high yielding wheat varieties. While diversity exists in the genetic resources and the elite germplasm pool, there in an increasing focus on developing efficient screening methodologies, faster generation advances and technologies such as the use of genomics and phenomics in varietal improvement. This chapter provides a detailed review of the major abiotic stresses in wheat, their effects on crop development, as well as breeding strategies and genomic technologies that are used or could be potentially used to develop stress tolerant wheat varieties.

2.2 Abiotic Stresses in Wheat

Abiotic stresses refer to the nonliving environmental factors that can affect the growth and development of the plant. In wheat, temperature (high/low), water stress and soil toxicity due to salinity or heavy metals are the abiotic stresses that known to affect productivity. Each of these stresses are further described in the following sections.

2.2.1 High Temperature

High temperature stress is a leading cause of reduction in wheat production globally, affecting both temperate and tropical wheat growing regions. Studies have reported significant losses in grain yield in the range of 6–10% for each Celsius rise in temperatures (Lobell et al. 2012; Asseng et al. 2015; Liu et al. 2016; Mondal et al. 2016b). High temperatures can affect almost every stage of the plant growth, from germination till grain filling, both morphologically and physiologically (Fig. 2.1). The timing and duration of the stress are important factors. For example, a 24-h heat treatment of wheat seedlings at 42 °C inhibited root and shoot development by increasing reactive oxygen and lipid peroxidase in the coleoptile and other developing organs (Savicka and Škute 2010). Physiologically, high temperature affects photosynthesis, activities of enzymes such as Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo), respiration, fluidity of thylakoid membrane, mobilization of water-soluble carbohydrates (WSC), seed proline content and cell hydration (Djanaguiraman et al. 2018; El Habti et al. 2020). High temperatures at the early vegetative stage can damage the photosynthetic machinery and cause oxidative damage of the cell membrane, which, in turn, leads to shortening of the vegetative period and reduced tillering (Fahad et al. 2017; Djanaguiraman et al. 2018). It has been reported that phenological events of wheat like booting, heading and physiological maturity advanced at a rate of 0.1 days to 0.31 days per year, due to preseason or seasonal temperature advancement (Ren et al. 2019). High temperatures at anthesis can lead to reduced pollen fertility, abnormal ovary development, and poor fertilizationthus reduction in seed set (Fahad et al. 2017). In northwestern wheat growing regions in India, temperatures above 34 °C during grainfilling, can hasten senescence significantly, thereby affecting yield (Lobell et al. 2012). High temperatures can reduce the grainfilling period by 45–60%, limiting the supply of photossimilates to grain, there by affecting grain yield significantly (Yang et al. 2002; Shah and Paulsen 2003; Mondal et al. 2016b).

Stress tolerant genotypes alleviate the effect of heat stress through various biochemical and physiological changes such as increase chlorophyll content, proline content, soluble sugar, and superoxide dismutase (SOD) activity. All these changes were found to be associated with improved kernel weight (Sattar et al. 2020). In

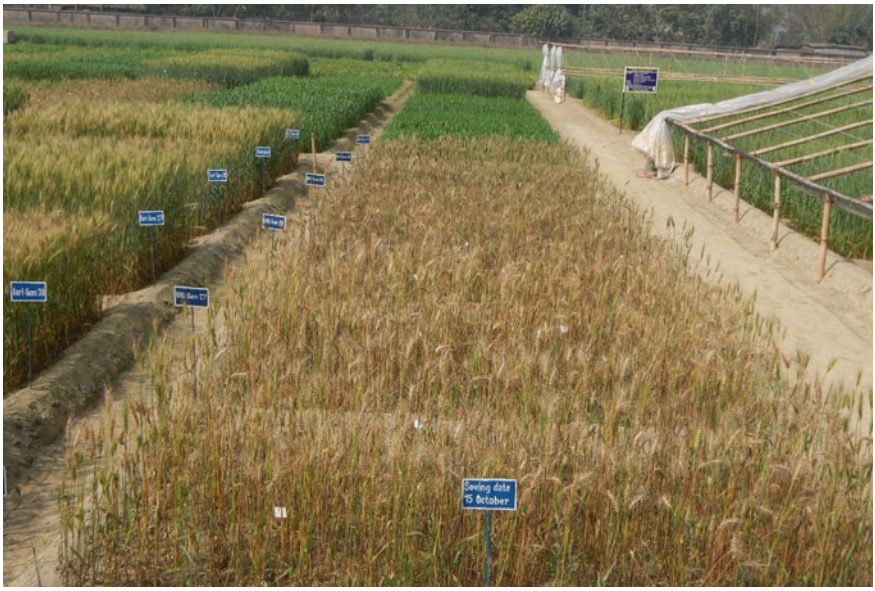

Fig. 2.1 Evaluating heat tolerance in early sowing at Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur, Bangladesh *Photo Credit* Farhad Nabin

barley, proline content increased under stress and showed an significant association with grain yield per spike ($r = -0.61^{**}$, $p > 0.01$) under heat stress (Sallam et al. 2018). Wheat stem characters also support grain filling under high temperatures through the increased mobilization of stem reserves, which contributes to grain yield. Internode length, stem diameter and density were associated with improving resource mobilization under heat stress. Under high temperature stress, production of antioxidants and heat-shock proteins has also been associated with tolerance, which enable the plants to maintain water potential and membrane integrity.

2.2.2 Drought

Drought stress results from reduction in water availability and can lead to considerable alterations in biochemical, physiological and molecular processes which affect plant growth and development (Fig. 2.2). Uneven rainfall patterns have increased the drought occurrences globally. It is projected that by 2040, 40% of the global wheat area will be subjected to severe water scarcity (Trnka et al. 2019). Like heat stress, drought can also affect the growth stages of the crop. Drought stress can occur alone or can also be associated with high temperature, soil salinity, high irradiance, wind and biotic stresses, which together can cause severe yield reductions (Wang et al. 2017).

Fig. 2.2 Drought stress in field plots at the Obregon station of International Maize and Wheat Improvement Center (CIMMYT) *Photo Credit* Uttam Kumar

Drought stress reduces tillering ability, plant height and leaf area in vegetative stages, whereas, at flowering it impairs pollination resulting in reduced spike fertility and grain number at grain filling (Farooq et al. 2014). The upper and lower spikelets of an ear are affected most by water stress. Drought during the grain filling stages, affects the enzyme activity involved in starch synthesis, causing reduced grain size and quality (Wardlaw and Willenbrink 2000; Ahmadi and Baker 2001). The rate and efficiency of photosynthesis also declines rapidly under drought stress. This is mainly due to decreased diffusion of carbon dioxide owing to the closure of stomata caused by loss of cell turgor. Further effects on the photosynthetic machinery are include reduction in the efficiency of photosystem II, reduction in the activity of photosynthetic enzymes, degradation of chloroplast structure, inhibition of chlorophyll biosynthesis and photo-oxidative damage to chlorophyll (Sallam et al. 2019). Therefore, photosynthetic rate and chlorophyll content can be used for drought tolerance screening (Dawood et al. 2019). Relative water content (RWC), a plant water status indicator, is found to be decreased in drought susceptible genotypes, and plants that can maintain the RWC are able to tolerate drought stress better (Allahverdiyev 2015). Plants tolerant to drought stress also displayed an increased excised leaf water retention (ELWR). ELWR measures the water retention mechanism in the stressed leaf (Lonbani and Arzani 2011). Leaf rolling, a

common phenomenon observed under drought, increases ELWR by reducing the exposed leaf surface area. Leaf waxiness and trichome density also play a key role in preventing excess water loss from the plant surface. Genotypes exhibiting these traits can minimize the effect of water stress (Guo et al. 2016).

Drought negatively impacts plant ionic homeostasis which results in reduced availabilityof major and minor nutrients (Sallam et al. 2019). The root volume decreases in dry soils which affects the nutrient uptake in plants (Farooq et al. 2012). Oxidative damage in spikes may occur due to rise in the levels reactive oxygen species (ROS) under severe drought stress, whichmodifies the cellular redox potential, resulting cell death (Caverzan et al. 2016). Secretion of antioxidant enzymes, namely catalase, SOD, and others has been reported to increase in drought tolerant genotypes under stress, as these antioxidant enzymesscavenge excess ROS and prevent cellular damage (Hasheminasab et al. 2012; Caverzan et al. 2016; Amoah et al. 2019; Laxa et al. 2019). Osmolyte accumulation during water stress enables the cells to minimize dehydration and preserve membrane structural integrity. Wheat plants under drought stress secrete other low-molecular-weight organic and inorganic solutes such as sugars, amino acids and polyols, which act as compatible osmolytes and facilitate the maintenance of cell turgor by decreasing osmotic potential (Loutfy et al. 2012). Some examples of the osmolytes involved in wheat drought response include proline, trehalose and glycine betaine (Iqbal 2018). Plant growth hormones are also known to regulate the responses to drought stress (Kaur et al. 2016). Abscisic acid (ABA) is known to mediate drought stress responses is well established. ABA regulates stomatal closure, promotes root growth and modifies leaf growth and elongation during drought stress (Xu et al. 2013).

2.2.3 Frost

Cold/frost stress is a major problem in temperate high latitude and altitude regions having extreme day and night temperature variation or even seasonal temperature variation. Temperatures below −5 °C for few days can cause extensive damage to vegetative tissue which is sometimes termed as frostbite (Skinner and Garland-Campbell 2008). It devastates wheat plants at the seedling and flowering stages. In case of less severe frosts, a light color stripe appears on vegetative leaves and gradually disappears when the leaves grow up. This reduces photosynthesis, and extended colder days (normally 1–2 days) may stop the growth completely (Nuttall et al. 2019). Severe low temperature during anthesis or early grain filling can result in a discolored spike with no seed. Rawson and Macpherson (2000) reported that low temperatures for three or more consecutive nights between Zadok's stage 4.9 to 5.9 can cause sterility in spring wheat.

Wheat plants were found to resist frost stress by making important physiological changes. Accumulation of osmolytes and unsaturated fatty acids as cryo-protectants to protect cells against freezing and water loss. The increase of unsaturated fatty

acid keeps leaves more fluid during the stress (Ruelland and Zachowski 2010) and hence increases frost tolerance in wheat (Cheong et al. 2020). Additionally, leaf water content is an important factor. Genotypes carrying higher leaf water content before the frost tend to have increased freezing effects due to ice formations. Consequently, the ice crystalspenetrate the cell causing severe damage to wheat leaves.

Like high temperatures, freezing temperatures occur in waves and cannot be predicted under field conditions. Improving frost tolerance in breeding programs is a very hard task due to the unpredictability of frost events in the target environments. Unfortunately, few studies have focused on frost tolerance and winter hardiness in wheat compared to studies on drought and heat stresses. While screening in a growth chamber could be advantageous in maintaining uniform testing conditions, it has been argued that it rarely simulates the actual field conditions. Recently, Cheong et al. (2019) utilized metabolomics and lipidomics of flag leaves to screen for genetic variation in frost tolerance of wheat. Their study suggested that metabolomics and lipidomics could distinguish between susceptible and tolerant germplasm and could be used explore the genetic variation in frost tolerance (Cheong et al. 2020). Due to lack of proper screening methodologies and the uncertainty of frost occurrences, breeding for frost tolerance gets little attention. A lot of research gaps still need to be addressed to develop frost tolerant varieties.

2.2.4 Salinity

Throughout the history of agriculture, soil salinity has been a major global agricultural threat affecting productivity and sustainability. Salinity can occur by both natural and human induced activity in all climatic conditions. In general, soil salinity is common in regions with insufficient rainfall that causes little or no leaching of mineral salts out from the rootzone. However, soil salinity is not confined to only arid or semi-arid regions; rather it is spreading to a wide range of hydrological and physiographic conditions. Globally, 20% of agricultural lands are saline and even the area and intensity of salinity are increasing (Miransari and Smith 2019). As a crop species, grain yield of wheat starts to reduce at a salinity level of 6–8 dS/m (Royo and Abió 2003).

Sodium chloride (NaCl) is considered the most soluble salt which can accumulate as sodium ion (Na^+) in wheat leaves. The high accumulation of Na^+ hinders the uptake of the essential micronutrients such as potassium (K^+) and calcium which are required for growth and development (Véry and Sentenac 2003). A high K^+/Na^+ ratio is key to reducing the effects of salt stress. While screening for salt tolerance is possible, there are several issues that tend to affect the screening process, such as (1) difficulty to separate pH effects from Na toxicity, (2) inability to control field soil composition, (3) increase in toxicity due excess salt increases while stabilizing pH (Singh et al. 2002). To address this, a new screening method was suggested using Na^+ humate as a surrogate for sodicity (Genc et al. 2016). This

method resolved the above problems and was also successfully used to assess the genetic variation for Na^+ exclusion in wheat.

Under salt stress, plants synthesize and accumulate organic compatible solutes or osmolytes, which act as osmoprotectants to maintain vital functions in osmotic adjustments, stabilizing proteins and membranes (Sharma et al. 2019). Application of plant hormones (abscisic acid, auxin, cytokinin, brassinosteroid, and gibberellic acid) or plant nutrients (potassium, phosphorus and calcium sulphate) can alleviate the effects of salinity stress (Kaya et al. 2009; Fahad et al. 2015).

Like frost tolerance, efforts on salt tolerance in wheat are few. Studies are needed to understand the morpho-physiological changes and the genetics underlying the salinity tolerance. Despite the minor ongoing breeding efforts in salinity tolerance, some salt tolerant varieties have been reported in the literature, such as W4909, W4910, Kharchia-65 and kri-210 (Kumar et al. 2015).

2.2.5 Heavy Metals

Chromium, cadmium, nickel, and lead are examples of heavy metals that tend to persist in the soil causing a reduction in the quality of the wheat crop. The accumulation of these heavy metals in plants is risky for human health as well (Simeonov et al. 2003). The main source of the accumulation in the soil is the irrigation with wastewater. The long-term use of such irrigation contaminates crops. The uptake of different heavy metals in wheat varies in different parts, with grains being reported to having the highest accumulation (Hassan et al. 2013). Many methods, such as oil replacement, chemical washing, chemical stabilization/immobilization, electro-kinetic extraction, and phytoremediation, have been suggested to reduce the effect of heavy metal accumulations. However, the effect of stress cannot be removed completely (Li et al. 2019). Cadmium and lead were reported to be the highest concentrated heavy metals in the kernels of wheat plants irrigated with sewage water (Hassan et al. 2013). It is very important to reduce the heavy metals content in wheat parts, especially grains, to reduce its effect on human health. Developing wheat cultivar having low uptake of heavy metals is the possible solution. Bread wheat has been reported to resist heavy metals using different mechanisms such as antioxidation and sequestration, exclusion, phytohormone and signal molecule regulation, and transcriptional regulation. There is little information and few studies as well on breeding for improving heavy metal resistance in wheat.

2.3 Wheat Adaptation to Stress Conditions

Wheat plants employ different strategies such as avoidance, escape or tolerance to adapt to abiotic stress conditions. Each of these strategies involve expression of various adaptive morpho-physiological and molecular changes in the plants. For

example, leaf rolling in response to drought/heat stress is common and considered an avoidance strategy. Through this leaf rolling the plant reduces light interception, transpiration and leaf dehydration thereby maintaining plant water status (Kadioglu et al. 2012). Adjusting phenology, is also an example of a plant avoidance strategy, where in at the perception of heat/drought stress, plants adjust their phenology to maximize the use of available resources for grain production. In a stress tolerance strategy, the plant mechanisms/traits have evolved over time to withstand the stress, for example, a deeper root system to access water from deeper layers of the soil in a water stress environment or maintaining membrane integrity under high temperature stress. Breeding for stress adaptation in wheat, involves integrating traits that enable stress adaptation and tolerance. The traits can either be sourced from the genetic resources, such as utilizing the primary and secondary genepools, or combining stress-adapted elite cultivars to improve grain yield and stability under abiotic stress.

2.4 Genetic Diversity/Resources for Adaptive/Tolerance Genes/Traits

Wheat genetic resources represent the existing genetic diversity that is critical to sustaining global wheat production. It includes sources for resistance to diseases and pests and tolerance to a wide range of abiotic challenges (Hoisington et al. 1999). Most high yielding wheat cultivars have genes or gene combinations bred by breeders from well-adapted cultivars following the best × best crossing approach (VanGinkel and Ortiz 2018). A study Crespo-Herrera et al. (2017) with germplasm of International Maize and Wheat Improvement Center (CIMMYT) has shown that genetically diverse germplasm has contributed to the increased genetic gains for grain yield. However, introgression of additional variation found in undomesticated wild species, landraces, and synthetics is necessary to address climate change and further improve wheat. A recent genetic analysis of 80,000 wheat accessions in the CIMMYT gene bank revealed unexplored genetic diversity within these accessions (Sansaloni et al. 2020).

2.4.1 Wheat Gene Pools

Wheat originated in the Fertile Cresent 10,000 years ago through the natural hybridization of two diploid wild grasses that produced tetraploid wheat (*Triticum turgidum* L. var. durum Desf. $2n = 4x = 28$). This tetraploid wheat further hybridized with goat grass (*Aegilopsis* spp.) to form hexaploid bread wheat (*Triticum aestivum* L. emThell. $2n = 6x = 42$) (Marcussen et al. 2014). A large genetic diversity exists within the *Triticum* species in different landraces, cultivars, and wild species as well as in different genepools of the tribeTriticeae (Hammer and Knüpffer 2015).

The primary gene pool consists of genetic species that share a common genome but were isolated from the mainstream (cultivated, wild and weedy forms) crop species. Landraces from Iran, Mexico, Turkey, Pakistan, India have over the years contributed to variety development due to easy transfer of genes within the primary gene pool. The secondary gene pool includes the closely related genomes, from where the gene transfer is difficult and primarily inter-specific hybridization is used. For example development of synthetic wheat, wherein, the hexaploid wheat could bere-synthesized by crossing the tetraploid durum with the *Aegilopsis tauschii* (Mujeeb-Kazi et al. 1996). It has been reported that genomic regions in *Ae. Tauschii* can increase grain weight up to 10% and contribute to higher grain yields (Röder et al. 2008). Synthetic wheat is a resource for both abiotic and biotic stress traits (Pradhan et al. 2012; Ogbonnaya et al. 2013; Morgounov et al. 2017; Naz et al. 2019). Synthetic wheat lines with tolerance to freezing, high temperatures, increased water uptake and water use efficiency have also been reported (Maes et al. 2001; Villareal et al. 2001; Yang et al. 2002; Reynolds et al. 2007). A recent study revealed that 20% of the advanced wheat lines distributed by CIMMYT's global wheat program, have synthetics in background (Rosyara et al. 2019). In the tertiary gene pool, gene transfer is difficult and not possible naturally. More than 0.8 million accessions of *Triticum* ssp., *Aegilops* ssp. and X *Triticosecale* are available worldwide. CIMMYT (>170,000 accessions) and ICARDA (>40,000 accessions) together hold more than 0.2 million wheat accessions, and these accessions are being utilized through different pre-breeding approaches. However, considerable duplications may exist among these accessions and the degree of duplication is difficult to assess without a global wheat genetic resource database (Singh et al. 2019b).

2.4.2 *Pre-breeding to Incorporate Stress Adaptation/ Tolerance*

Agronomic and genetic characterization of the gene bank material through various multi-disciplinary consortiums such as Heat and Drought Wheat Improvement Consortium (HeDWIC, http://www.hedwic.org/), Seeds of Discovery (https://seedsofdiscovery.org/) and International Wheat Yield Partnership, (IWYP, https://iwyp.org/), is the best way to use high value alleles (Fig. 2.3). Detailed genetic and phenotypic characterization could also enable the use of predictive approaches like genomic prediction (Yu et al. 2016). Several approaches have successfully been used to incorporate valuable alleles into elite germplasm. Complementary strategic crossing by dissecting grain yield into component characters is one approach (Reynolds and Langridge 2016; Reynolds et al. 2017). Selection of these segregating generations through high throughput phenotyping (Tattaris et al. 2016; Singh et al. 2019a) and speed breeding are alternative ways for generation advancement (Watson et al. 2017).

Fig. 2.3 Screening of Pre-breeding materials at Obregon station of the International Maize and Wheat Improvement Centre (CIMMYT) *Photo Credit* Sivakumar Sukumaran

2.5 Breeding Approaches to StressAdaptation/Tolerance

Breeding approaches are designed to improve several traits simultaneously for stress tolerance. Farmers generally prefer varieties that produce stable and high yields under the ever-changing climatic conditions. Thus, breeders must not only focus on increasing grain yield, but stability as well. Considerable genetic variation exists in the elite wheat germplasm that enable breeders to screen, identify and cross between elite germplasm to improve productivity. However, the key bottlenecks that remain are; effective screening environments/techniques for stress and deploying efficient breeding strategies to reduce the cycle time required to develop such stress tolerant varieties.

Screening environments are key for breeding programs selection efficiency. Screening in early generations under stress conditions could be ideal for selecting stress tolerant lines; however, they may lack the stability to perform across years and climatic ranges. Breeding programs evaluate their materials in target environments where high temperature stress or drought stress is the major issues; however, often due to uncertainty in weather patterns the stress is not uniform. This is particularly more of an issue for stresses such as frost or salinity, which are highly unpredictable and heterogenous. For high temperature stress screening, a common

methodology used by the breeding programsis to sow on multiple dates to expose the lines to stress. Some research programs use temporary heat tents to introduce short term high temperature stress; however, this method may be an expensive exercise when screening breeding populations and may be more suitable for limited screening of germplasm/elite lines for stress tolerance (Hein et al. 2019). Drought stress can be applied by controlling irrigation application, however, in the rainfed regions, this becomes a problem due to uncertainty in rains. Temporary rainout shelters are option used by various research programs (Hoover et al. 2018) and could work in later yield testing stages of the breeding program but may not be suitable during early generation selection. Conversely, use of drip irrigation is also suggested for drought screening to have a better control of the timing and amount of water applied for drought screening (Arafa et al. 2009; Habbasha and Ramadan 2014). Another essential component of selection efficiency is also to consider a statistical design to account for the spatial or annual variation while screening for abiotic stress tolerance. Use of multilocation and multiyear trials at targeted locations in yield testing stages will enable the identification of stress tolerant germplasm.

Strategies to improve cycle time, a key component in development of stress tolerant varieties, are required by the breeding programs to accelerate the variety development process. Inthelate 1940s, Dr. Norman Borlaug initiated an unorthodox method of shuttle breeding that went onto change wheat breeding strategies globally. The ability to grow and select wheat populations in two diverse locations/seasons/year in Mexico, made it possible to advance wheat generations faster compared to standard breeding programs (Crespo-Herrera et al. 2017, 2018; Mwadzingeni et al. 2017; Mondal et al. 2020). The contrasting environments and day lengths at the two locations (Toluca with cool temperature and high rainfall and Ciudad Obregón with a warm irrigated environment) enabled Dr. Borlaug and CIMMYT breeders to develop germplasm with broad adaptation and yield stability globally. Therefore, shuttle breeding became the cornerstone of the Green Revolution. While not all breeding programs are not able to follow a shuttle breeding scheme, an alternate could be faster generation advancement using a screen houses or greenhouse. Studies have shown that in temperature and light controlled greenhouses, six generations/cycles of wheat can be grown which could significantly contribute to shortening generation advancement (Ghosh et al. 2018; Watson et al. 2018). The rice breeding program at the International Rice Research Institute (IRRI), Philippines have implemented such a strategy and have improved resource allocation in the line development process (Cobb et al. 2019). CIMMYT's wheat breeding program is also looking forward to implementing a rapid generation advancement scheme in a field-based screen house to further accelerate varietal development. Accelerating breeding cycles can be the most efficient way to increase genetic gains in grain yield under abiotic stress; however, it has remained under-exploited. Implementing genomic estimated breeding values in selection could significantly improve the breeding approaches.

2.6 Molecular Mapping for Abiotic Stress Tolerance

A significant number of Quantitative trait loci (QTL) mapping studies to detect stress tolerant QTLs using biparental populations have been reported in wheat. For the sake of brevity, we have summarized below the findings of QTL mapping done for the most important abiotic stresses: drought, heat and salinity.

2.6.1 QTLs for High Temperature Stress

The advances in understanding the underlying genetics of heat tolerance is noteworthy. Initial studies started in the substitution lines of tetraploid wheat cultivar Langdon to map heat tolerance genes (Sun and Quick 1991). In the later years, substitution lines of cultivars Chinese Spring and Hope were used for mapping (Ruqiang et al. 1996). The results from mapping using substitution lines show that chromosomes 3A and 3B frequently have genes controlling heat tolerance. Over the past decades, QTL mapping studies have investigated the genetic basis of heat tolerance using biparental populations and many of these studies consistently reported QTL hotspots on chromosome 3B. For example, QTLs for grain yield, thousand grain weight, quantum efficiency of photosystem and canopy temperatures have been reported on chromosome 3B (Mason et al. 2010a; Bennett et al. 2012a; Mondal et al. 2015b; Sharma et al. 2017). QTL hotspots have been also been identified on chromosomes 2D (30 QTLs), 5A (29 QTLs), 7D (19 QTLs), 7A (18 QTLs), 1B (17 QTLs), 2B (16QTLs), 4A and 5B (15 QTLs) each (Mason et al. 2010b, 2013; Vijayalakshmi et al. 2010; Bennett et al. 2012b; Paliwal et al. 2012; Tiwari et al. 2013; Talukder et al. 2014; Mondal et al. 2015a; Bhusal et al. 2017; Sharma et al. 2017). Utilization of heat susceptibility index has been common in many of above cited QTL mapping studies (Mason et al. 2010b, 2013; Paliwal et al. 2012; Tiwari et al. 2013; Talukder et al. 2014; Bhusal et al. 2017).

2.6.2 QTLs for Drought Stress

Similar to heat stress, a mapping QTLs for traits associated to drought tolerance has also be been considerable (Gupta et al. 2017; Tshikunde et al. 2019). Many of these investigations have targeted physiological traits, the most common being canopy temperature, chlorophyll content, carbon isotope discrimination, relative water content, water-soluble carbohydrates, photosynthetic capacity/rate, cell membrane thermostability and various root traits such as root elongation rate, primary and lateral root length, root angle, deep root ratio, root to shoot ratio, root biomass and weight (Rebetzke et al. 2008; Pinto et al. 2010; Hamada et al. 2012;

Mohammady et al. 2012; Bai et al. 2013; Bharti et al. 2014; Czyczyło-Mysza et al. 2014; Ayalew et al. 2016; Voss-Fels et al. 2018). Bai et al. (2013) reported co-location of QTL for root component traits with plant height QTL, thus suggesting that selection for shorter plants has affected root growth and the ability to withstand drought. Soriano and Alvaro (2019) conducted a meta-analysis for root-related traits, which projected 634 QTLs on a consensus map having 7352 markers. They identified 94 consensus root metaQTL (MQTL) and for 68 of them, gene models were found related to root architecture and/or drought stress response.

Stay-green trait (i.e. delayed foliar senescence character) has also been associated with adaptation to drought stress. Stay-green helps the plant to continue photosynthesizing under stress by maintaining greenness (Borrell et al. 2014; Thomas and Ougham 2014). A few mapping studies have indicated that breeders selecting for short-height, non-lodging, productive wheat varieties have simultaneously selected for the stay-green and day length insensitivity. Among such evidences is a QTL for delayed flag leaf senescence reported close to an allele of the *PpdD1*on chromosome 2D and the height gene*Rht8* (Pestsova and Röder 2002; Verma et al. 2004). Reflectance based measurements, such as normalized difference vegetation index (NDVI), is associated with stay-green and grain yield under drought conditions (Babar et al. 2006a, b; Hazratkulova et al. 2012; Shi et al. 2017; Liu et al. 2019). Studies have reported association between grain yield with NDVI estimated in both early growth stages and grain filling stages (Babar et al. 2006b; Hazratkulova et al. 2012). Shi et al. (2017) mapped major QTL for NDVI on chromosome 5A with a maximum PVE of 20.21% and identified pleiotropic QTLs for agronomic and stay-green traits on chromosomes 1B, 3D, 4D and 7A. Such consistent QTLs for NDVI, biomass and yield components could enable the use of these traits as an indirect selection criterion for grain yield improvement.

Various studies have utilized indices for mapping drought tolerance. A drought sensitivity index is one such index that is used to identify QTLs for drought tolerance in mapping studies (Denčić et al. 2000; Foulkes et al. 2007; Semenov and Halford 2009; Alexander et al. 2012; Chopra et al. 2012; Gahlaut et al. 2017). Gahlaut et al. (2017) utilized a double haploid population to investigate nine drought responsive traits across 22 environments in India under both irrigated and rainfed conditions. The authors identified QTL for DSI for each of the nine traits and reported five major QTLs with PVE of ~20% on chromosomes 5A and 7A (*QDa.ccsu-5A.2*, *QDm.ccsu-5A.2*, *QDa.ccsu-7A*, *QDm.ccsu-7A* and *QGfd.ccsu-7A*), of which four were identified in multiple environments.

Studies have also reported genomic regions harboring common QTLs for tolerance to both heat and drought (Pinto et al. 2010; Bennett et al. 2012b; Chopra et al. 2012; Liu et al. 2019). Acuña-Galindo et al. (2015), in their meta-analysis study with QTL information for 81 distinct traits assembled from 30 different studies identified 66 MQTL regions distributed throughout the genome of which 20 were specific to drought and two to heat stress tolerance, while 43 MQTLs on chromosomes 1B, 2B, 2D, 4A, 4B, 4D, 5A, and 7A were associated to both heat and drought tolerance.

2.6.3 Genome-Wide Association Studies for Abiotic Stress Tolerance

The development of single nucleotide polymorphism (SNP) marker technologies from different sequencing platforms, genetic marker tool kit has undergone a remarkable shift in wheat. The availability of thousands of SNPs has led to the use of genome-wide association (GWAS) approach to dissect traits in wheat. GWAS uses a linkage disequilibrium approach to identify association between genetic loci and traits and bypasses the need to generate mapping panels, thus providing a powerful alternative to linkage mapping (Sukumaran and Yu 2014; Sehgal et al. 2017).

Extensive research on candidate gene (CG)-based association mapping and GWAS approaches has led to identification of genomic regions associated to drought stress tolerance (Khadka and Raut 2011). Investigations have targeted grain yield, yield components (Alexander et al. 2012; Ahmad et al. 2014; Sukumaran et al. 2015; Sehgal et al. 2017; Afzal et al. 2019) and/or combination of yield and physiological traits (Zhang et al. 2013; Edae et al. 2013, 2014; Ain et al. 2015; Gahlaut et al. 2017; Bhatta et al. 2018; Lehnert et al. 2018; Qaseem et al. 2018; Afzal et al. 2019; Liu et al. 2019; Molero et al. 2019). Edae et al. (2013) conducted CG-based association mapping in wheat and reported associations of SNPs in three CGs, *DREB1A*, ERA1 and *1-FEH* with multiple agronomic and physiological traits. In another CG-based association mapping study in wheat, the *TaSnRK2.8* gene (an SNF-1 type serine-threonine protein kinase) was linked to flag leaf width, plant height and water-soluble carbohydrates under drought conditions (Zhang et al. 2013). In the past few years, root architectural traits (mainly root length, biomass and root/shoot dry weight ratio) have been extensively investigated by GWAS under drought stress (Wang et al. 2013; Ayalew et al. 2016; Dar et al. 2017).

A comprehensive analysis of GWAS publications in wheat reveal frequent marker-trait associations (MTAs) on chromosome 4A for drought-stress tolerance (Alexander et al. 2012; Edae et al. 2014; Sehgal et al. 2017). For example, Edae et al. (2014) reported mapping of DSI and other leaf traits on chromosome 4A while Sehgal et al. (2017) identified two QTLs stable across irrigated and stress environments on chromosome 4A for grain yield.

To fully exploit the potential of dense genome-wide SNPs available from SNP arrays and other high density genotyping platforms in wheat (9K, 90K, 660K and 820K SNP arrays, genotyping-by-sequencing and DArTseq), the latest investigations have explored a haplotypes-based GWAS approach for identifying stable QTLs (Bhatta et al. 2018; Afzal et al. 2019; Sehgal et al. 2020).

Heat and salinity tolerance using the GWAS approach has received increased attention in last couple of years (Sehgal et al. 2017, 2020; Maulana et al. 2018; Oyiga et al. 2018; Qaseem et al. 2018; Schmidt et al. 2020). Maulana et al. (2018) were the first to report MTAs for seedling heat tolerance in wheat. Furthermore, underlying candidate genes for heat tolerance QTL on chromosomes 3B and 4B were identified in this study. Sehgal et al. (2020) reported 15 stable haplotype

blocks associated with grain yield under heat stress in CIMMYT spring wheat germplasm. Qaseem et al. (2018) and Schmidt et al. (2020) identified common QTLs for heat and drought tolerance using GWAS in 108 and 315 spring wheat accessions, respectively. While, Qaseem et al. (2018) reported stable associations on chromosome 5A and 7D, Schmidt et al. (2020) identified the significant association on chromosome 6A for combined heat and drought tolerance. This 6A locus was associated with increased grain weight, thousand-kernel weight, grain number and grain number under drought and heat stress.

Yu et al. (2020) identified three haplotypes for salt tolerance index on chromosomes 1A (*QSt.nwafu-1A*), 3B (*QSt.nwafu-3B*) and 6B (*QSt.nwafu-6B*) in a panel of 307 wheat accessions, including local landraces, historical and cultivated varieties using an Affymetrix wheat 660K SNP array for salt tolerance. The QTL on chromosome 6B was reported to be novel. In another study on GWAS dissection of salt tolerance by Hu et al. (2020) reported QTLs for yield and other associated traits on chromosomes 4A, 5A, 5B and 7A for salt tolerance.

2.7 Genomic Selection in Abiotic Stress Adaptation/ Tolerance

A widely researched genomics assisted approach in selection or breeding for abiotic stress adaptation is genomic selection (GS). It utilizes the information from genome-wide markers to calculate genomic estimated breeding values (GEBVs; Meuwissen et al. 2001) for selecting individuals. Since GS requires whole genome markers, it captures both major and minor gene effects. GS, therefore, is more advantageous as compared to MAS as it bypasses the need to discover QTL for the target traits (Nakaya and Isobe 2012). It offers the potential to accelerate genetic gain by complementing selection accuracy and intensity of breeding cycles.

In wheat, extensive research has been conducted in the past few years with various statistical models, marker types and densities and by including pedigrees, environmental covariates and high-throughput imaging data for prediction of complex traits such as stress tolerance (Crossa et al. 2010, 2015, 2019; Heffner et al. 2011a; Cossani and Reynolds 2012; Poland et al. 2012; Burgueño et al. 2012; de los Campos et al. 2013; Pérez and de los Campos 2014; Rutkoski et al. 2015; Juliana et al. 2017; Kristensen et al. 2018; Sehgal et al. 2020). Various types of training populations such as bi- and tri-parental populations, multi-lines, multi-subpopulations, multi-families and gene bank accessions have been utilized (Crossa et al. 2010, 2016; Heffner et al. 2011a, b; Saint Pierre et al. 2016; Hoffstetter et al. 2016; Cericola et al. 2017; Muleta et al. 2017; Ornella et al. 2017; Joukhadar et al. 2017; Juliana et al. 2017; Dong et al. 2018; Ladejobi et al. 2019). These studies have led to the following important conclusions: (1) a larger training set results in a better prediction, (2) increased relationship between training and test populations leads to improved prediction accuracies, (3) incorporating G x E

interactions into GS models improves prediction accuracy, and (4) incorporating pedigree information in GS models is as effective as markers.

Regarding prediction for multiple environments or for erratic or unpredictable environments (low or no rainfall, drought stress, heat stress etc.), a significant breakthrough came through from the study of Burgueño et al. (2012). They reported that prediction models fitted with G × E interaction effects improve trait predictions significantly than univariate or single-environment prediction models. Later, several similar investigations on multi-location and multi-year data incorporated G x E effects, and reported substantial improvements prediction accuracies (Heslot et al. 2014; Jarquín et al. 2014; Crossa et al. 2015; Lopez-Cruz et al. 2015; Pérez-Rodríguez et al. 2017). Heslot et al. (2014) used environmental co-variables in addition to marker data in their G × E model and showed 11% improvement inprediction accuracy. A reaction norm model introduced by Jarquín et al. (2014) used high-dimensional random variance-covariance structures of markers and environmental covariates, which when applied for prediction of grain yield improved accuracy from 17 to 34% than that of models based on main effects model. This same strategy was used Pérez-Rodríguez et al. (2017) to predict the performance of CIMMYT wheat lines across South Asia. Lopez-Cruz et al. (2015) used another approach in three CIMMYT wheat data sets for predictions. In this approach, marker × environment interaction GS model was applied to estimate genomic values as main effects or steady across environments and interactive effects or within an environment. The prediction accuracies using this model were 5–29% higher.

Machine learning (ML) and deep learning methods are becoming popular and have attracted a lot of scientists today for conducting multiple analyses (González-Camacho et al. 2018). ML is a field of computer science that captures characteristics of target patterns using algorithms and information on existing samples (Gonzalez-Sanchez et al. 2014). Limited research using ML methods has been conducted for predicting yield for marginal environments (Pantazi et al. 2016). Three ML methods, counter-propagation artificial neural networks (CP-ANN), XY-fused networks (XY-F) and supervised kohonen networks (SKN), were tested by Pantazi et al. (2016) for predicting wheat yield. It utilized satellite-based data NDVI and soil parameters as inputs. The average overall accuracies were, 78.3, 81.65 and 80.92%, for the three models, CP-ANN, SKN and XY-F, respectively. The SKN model further outperformed when also used for a cross-validation-based yield prediction for the low, medium and high yield classes, with accuracies reported to 91, 70 and 83% accuracies, respectively.

A major area of research focus in past two years has been GS modelling by integration of major genes and/or stable loci identified in GWAS as fixed effects (Odilbekov et al. 2019; Sarinelli et al. 2019; Sehgal et al. 2020). Although there are more evidences of the success of this approach in predicting for disease resistance (Odilbekov et al. 2019; Sarinelli et al. 2019), limited research on prediction for abiotic stressed environments has shown encouraging results (Sehgal et al. 2020). Sehgal et al. (2020) used a prediction model that included fixed effects of the stable loci identified by a haplo typed-based GWAS approach. The authors reported a 9–10% increase in prediction accuracies with the model accounting for the stable loci as fixed effects.

With significant advancements being made in imaging platforms for high-throughput phenotyping (HTP), the integration of HTP data in GS models has gained a lot of attention in wheat (Rutkoski et al. 2016; Crain et al. 2018a; Juliana et al. 2019b). Rutkoski et al. (2016) investigated the incorporation of secondary traits, canopy temperature (CT) and green and red NDVI, in GS models for prediction of grain yield in five contrasting environments (well irrigated, drought and heat stress). The authors modeled CT and NDVI on training and test sets, and grain yield on the training set in a multivariate analysis and observed 37% improvement in prediction accuracy after correcting for days to heading. Crain et al. (2018b) utilized over 1.1 million phenotypic data points on 1170 advanced CIMMYT lines in drought and heat stress environments. The secondary traits were modelled as a response in multivariate models or as a covariate in univariate models. From seven to 33% increase in prediction accuracy above the standard univariate model was reported by the authors. A multivariate prediction of grain yield in drought and heat stress environments by Juliana et al. (2019b) reported prediction accuracies of 0.56 and 0.62, respectively.

2.8 The New Breeding Technology—CRISPR/Cas Mediated Genome Editing

The genome editing technology using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) has transformed plant molecular biology in the last few years. It is a versatile and powerful tool that can create targeted point mutation or indels to change the functional nature of a gene and can even replace a faulty gene with a corrected coding sequence (Biswal et al. 2019). Native promoters can be modified appropriately to modulate the gene expression or can be replaced with a different promoter.

Plant abiotic stress tolerance is controlled by complex regulatory networks (Sreenivasulu et al. 2007). Genetic redundancy due to polyploidy has hindered molecular breeding efforts aimed at gene discovery for abiotic stress tolerance in wheat. Though molecular biology of wheat lags that of other cereals, research on model plants has identified multiple genetic elements and response mechanisms to different abiotic stresses that can be exploited to build abiotic stress tolerance of wheat. The complicated nature of abiotic stress response pathways as well as consumer acceptance have greatly slowed the progress of genetic engineering of wheat for abiotic stress tolerance.

The CRISPR/Cas9 mediated genome editing offers a unique solution that combines the best features of conventional breeding and transgenic technology. The CRISPR tools are generally delivered into plant cells via plasmid vectors by genetic transformation. In some cases, the CRISPR tools have also been delivered as ribonucleo-protein (RNP) complexes that could enable transient editing of plant genes without the need for transgene integration into the host genome. This

"DNA-free" RNP complex has also been successfully applied in bread wheat (Liang et al. 2017). In either case, the CRISPR tools are not required after the generation of mutations and can be spontaneously removed by meiotic segregation. The final plants can be like the natural variants, free of any transgene scar. The donor lines of heterosis breeding are often associated with many negative agronomic traits that get transferred to the hybrid lines due to linkage drag. It takes several rounds of backcrossing to get rid of the unwanted genetic elements that makes the breeding process lengthy, resource and time demanding, and sometimes impossible. CRISPR/Cas9 mediated genome editing is versatile and precise in creating specific mutations at the target site. The CRISPR/Cas mediated gene edited lines can be released within 3–4 years without the need for backcrossing. The CRISPR/Cas mediated genome editing is now being referred to as the new breeding technology or advanced breeding technology that needs much less regulatory clearance than transgenic products in many countries (Genetic Literacy Project).

2.8.1 Example of Application in Abiotic Stress Tolerance

The CRISPR/Cas9 system has redefined the boundaries of biological research in the last few years. Generally, it comprises of a nuclease such as Cas9 or Cpf1 (CRISPR from *Prevotella* and *Francisella* 1) and the guide RNA (gRNA) that leads the nuclease to the target site. The Cas9 nuclease is guided by a short CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA), which anneal together to form the gRNA. Both crRNA and tracrRNA can even be combined as a single guide RNA (sgRNA) (Jinek et al. 2012). The crRNA identifies the target site by Watson-Crick base pairing with the template strand. The target recognition of Cas9 also requires a dinucleotide proto-spacer adjacent motif (PAM) immediately following the spacer sequence (Biswal et al. 2019). Therefore, any unique 20 nucleotides stretch preceding the PAM sequence can serve as the spacer sequence that can be transcribed into crRNA in plants. The Cas9 nuclease makes a double stranded break, which is immediately repaired by the cellular repair machinery generally through non-homologous end joining (NHEJ). The NHEJ often introduces, deletes or replaces one or a few bases. This results in a change in the protein sequence or, more often, the introduction of a frameshift mutation with a stop codon after few bases downstream of the target site. The resulting short peptide can be degraded by nonsense-mediated decay of the transcript (Shaul 2015). The target site can also be repaired or modified with a desired DNA sequence by homology-directed repair (HDR) when a homologous DNA template is provided. This can help replace a faulty gene or even to replace a promoter sequence for an appropriate level of gene expression. Recent improvements to the CRISPR/Cas system also offers to repress or enhance the expression of certain genes (La Russa and Qi 2015). By adding more than one gRNA into delivery vector, the CRISPR mediated genome editing can be applied to target multiple genes or homeologs at the same time (Ma et al. 2015; Xie et al. 2015).

One of the basic uses of the CRISPR/Cas system is to validate the gene function by knocking out the candidate genes. Theepidermal Patterning Factor like-9 (*AtEPFL9*) gene is linked early development in Arabidopsis is associated with stomatal development. Knocking out its rice orthologue (*OsEPFL9*) using CRISPR/Cas9 and CRISPR-Cpf1 approach resulted in an eightfold reduction of stomatal density on the abaxial leaf surface of the mutant rice plants (Yin et al. 2017). Genetic ablation of the MYB transcription factor *GmMYB118* in soybean validated its function in tolerance drought and salinity stress (Roca Paixão et al. 2019).

In maize, ARGOS8 serves as a negative regulator of ethylene responses. The replacement of the native *ARGOS8* promoter with the maize GOS2 promoter using CRISPR/Cas mediated genome editing resulted in higher expression of the *ZmARGOS8* gene (Shi et al. 2017). The higher expression led to lower ethylene sensitivity and higher maize yield. Arabidopsis histone acetyltransferase 1 (AtHAT1) switches the chromatin to a relaxed state, which promotes gene expression. When a catalytic core from AtHAT1 was fused to the *N*-terminal part of a dCas9 and that was targeted to the endogenous promoter of *AtAREB1* gene, the expression levels of *AtAREB1* and *AtRD29A*, a genes positively regulated by AREB1, were significantly increased (Roca Paixão et al. 2019). The CRISPR-activated (CRISPRa) lines showed rapid trigger of stomatal closure, higher chlorophyll content and better survival after exposure to drought stress. This experiment presents an ideal example of how the CRISPR/Cas system can selectively activate certain genes to make the plants resilient to environmental stresses.

Kim et al. (2018) demonstrated successful targeted editing of two stress-responsive transcription factor genes, dehydration responsive element binding protein 2 and wheat ethylene responsive factor 3, by transient expression of Cas9 and the guide RNA in wheat protoplast. Though wheat genetic transformation of elite wheat lines still remains a bottleneck in genome editing, a recent report indicates a substantial improvement in wheat transformation efficiency by the use of fusion proteins combining wheat growth-regulating factor (GRF) 4 and its cofactor GRF-interacting factor 1 (Debernardi et al. 2020).

2.8.2 Application in Generating Diversity

Genetic diversity forms the basis of plant breeding. Though *Triticeae* species are relatively rich in genetic diversity (Mondal et al. 2016a), genetic variation for certain traits is limited (Mishra et al. 2016). Domestication and modern plant breeding have further narrowed this diversity in land races and hybrid lines (Reif et al. 2005). Researchers have been opting for chemical mutagenesis such as ethyl methane sulfonate treatment, fast neutron bombardment and gamma irradiation and other methods to generate artificial mutants (Boyd et al. 2006; Mishra et al. 2016; Hong et al. 2019). However, these methods are limited by the random and undirected nature of the mutation. The genetic redundancy also forms a bottleneck to find mutants of all homeologs from natural germplasm as well as in the artificial

mutant population for functional validation of candidate genes. This problem can be easily overcome by CRISPR-mediated genome editing (Wang et al. 2014). The CRISPR-based base editors offer another elegant alternative to introduce local and genome-wide polymorphism that can serve as novel source of germplasm.

CRISPR/Cas mediated genome editing allows to precisely create SNPs in the target genetic element. Fusion of a cytidine deaminase with nuclease-inactive Cas9 (dCas9) enables the cytidine deaminase to convert the cytidine nucleotide to uracil at the target site (Mishra and Joshi 2020). The sgRNA guides the dCas9 to the target site where the dCas9 generates a short stretch of single-stranded DNA that can be the target of cytidine deaminase. This results in a mismatch (U · G) that is repaired by the cellular DNA-repair machinery to generate a U · A base pair which is subsequently converted to T · A (Gaudelli et al. 2017). An *E. coli* tRNA adenine deaminase has been engineered to convert Adenine to Inosine (Gaudelli et al. 2020). Inosine, like Guanine, base pairs with cytosine (I · C) that gets modified to G · C by cellular machinery. Thus, targeted sequence diversity in genes related to abiotic stress response pathway can be generated by the CRISPR/Cas system. A CRISPR/Cas9 mediated genome-wide mutagenesis screen was established using an sgRNA pool targeting almost all genes in rice (Lu et al. 2017). More than 90,000 T0 mutant rice lines were generated that can serve as an important resource for studying various gene functions. A similar strategy in wheat can help to characterize large number of wheat genes whose function are yet to be validated.

2.9 Summary

As wheat production continues to be challenged by the threat of abiotic stresses, wheat breeders/researchers continue to understand the effect and underlying genetic elements of these stresses to improve tolerance. While high temperature stress and drought stress have been extensively studied, other abiotic stresses such as frost and salinity still need more research and better breeding strategies. The advances in technologies, such as availability of annotated genome sequence, better and cheaper molecular markers, improved prediction models for genomic selection, and strategies to improve breeding efficiencies, place us in a unique position to deal with the upcoming challenges. It is important to evaluate these technologies either in experimental phases or as part of a breeding programs for optimization and effective application. While still new, CRISPR/Cas technology shows potential not only for building biotic resistance in wheat but also for abiotic stress tolerance. Continued investments in research and breeding are also key for improving knowledge of stress tolerance mechanisms, identifying their genetic regulation, breeding accuracy, and selection efficiency to develop stress tolerant, high yielding stable varieties.

References

Acuña-Galindo MA, Mason RE, Subramanian NK, Hays DB (2015) Meta-analysis of wheat QTL regions associated with adaptation to drought and heat stress. Crop Sci 55:477. https://doi.org/10.2135/cropsci2013.11.0793

Afzal F, Li H, Gul A et al (2019) Genome-wide analyses reveal footprints of divergent selection and drought adaptive traits in synthetic-derived wheats. G3 Genes Genomes Genet 9:1957–1973. https://doi.org/10.1534/g3.119.400010

Ahmad MQ, Khan SH, Khan AS, et al (2014) Identification of QTLs for drought tolerance traits on wheat chromosome 2A using association mapping. Intl J Agric Biol 16

Ahmadi A, Baker DA (2001) The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. Plant Growth Regul 35:81–91. https://doi.org/10.1023/A:1013827600528

Ain QU, Rasheed A, Anwar A et al (2015) Genome-wide association for grain yield under rainfed conditions in historical wheat cultivars from Pakistan. Front Plant Sci 6:743. https://doi.org/10.3389/fpls.2015.00743

Alexander LM, Kirigwi FM, Fritz AK, Fellers JP (2012) Mapping and quantitative trait loci analysis of drought tolerance in a Spring wheat population using amplified fragment length polymorphism and diversity array technology markers. Crop Sci 52:253. https://doi.org/10.2135/cropsci2011.05.0267

Allahverdiyev T (2015) Effect of drought stress on some physiological traits of durum (*Triticum durum* Desf.) and bread (*Triticum aestivum* L.) wheat genotypes. J Stress Physiol Biochem 11:29–38

Amoah JN, Ko CS, Yoon JS, Weon SY (2019) Effect of drought acclimation on oxidative stress and transcript expression in wheat (*Triticum aestivum* L.). J Plant Interact 14:492–505. https://doi.org/10.1080/17429145.2019.1662098

Arafa Y, Wasif EA, Mehawed HE (2009) Maximizing water use efficiency in wheat yields based on drip irrigation systems. Aust J Basic Appl Sci 790–796

Asseng S, Ewert F, Martre P et al (2015) Rising temperatures reduce global wheat production. Nat Clim Chang 5:143–147. https://doi.org/10.1038/nclimate2470

Ayalew H, Liu H, Yan G (2016) Quantitative analysis of gene actions controlling root length under water stress in spring wheat (*Triticum aestivum* L.) genotypes. Crop Pasture Sci 67. https://doi.org/10.1071/CP15244

Babar MA, Reynolds MP, Van Ginkel M et al (2006a) Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. Crop Sci 46:1046. https://doi.org/10.2135/cropsci2005.0211

Babar MA, Reynolds MP, Van Ginkel M et al (2006b) Spectral reflectance indices as a potential indirect selection criteria for wheat yield under irrigation. Crop Sci 46:578. https://doi.org/10.2135/cropsci2005.0059

Bai C, Liang Y, Hawkesford MJ (2013) Identification of QTLs associated with seedling root traits and their correlation with plant height in wheat. J Exp Bot 64:1745. https://doi.org/10.1093/jxb/ert041

Bennett D, Reynolds M, Mullan D et al (2012a) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments. Theor Appl Genet 125. https://doi.org/10.1007/s00122-012-1927-2

Bennett D, Reynolds M, Mullan D et al (2012b) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments. Theor Appl Genet 125:1473–1485. https://doi.org/10.1007/s00122-012-1927-2

Bharti S, Balyan HS, Gupta P (2014) Quantitative trait loci analysis for some root traits in bread wheat (*Triticum aestivum* l.). Intl J Agric Sci 4:214–221

Bhatta M, Morgounov A, Belamkar V, Baenziger PS (2018) Genome-wide association study reveals novel genomic regions for grain yield and yield-related traits in drought-stressed synthetic hexaploid wheat. Intl J Mol Sci 19:3011. https://doi.org/10.3390/ijms19103011

Bhusal N, Sarial AK, Sharma P, Sareen S (2017) Mapping QTLs for grain yield components in wheat under heat stress. PLoS ONE 12:e0189594. https://doi.org/10.1371/journal.pone.0189594

Biswal AK, Mangrauthia SK, Reddy MR, Yugandhar P (2019) CRISPR mediated genome engineering to develop climate smart rice: challenges and opportunities. Semin Cell Dev Biol 96:100–106. https://doi.org/10.1016/j.semcdb.2019.04.005

Borrell AK, van Oosterom EJ, Mullet JE et al (2014) Stay-green alleles individually enhance grain yield in sorghum under drought by modifying canopy development and water uptake patterns. New Phytol 203:817. https://doi.org/10.1111/nph.12869

Boyd LA, Smith PH, Hart N (2006) Mutants in wheat showing multipathogen resistance to biotrophic fungal pathogens. Plant Pathol 55:475–484. https://doi.org/10.1111/j.1365-3059.2006.01402.x

Burgueño J, de los Campos G, Weigel K, Crossa J (2012) Genomic prediction of breeding values when modeling genotype × environment interaction using pedigree and dense molecular markers. Crop Sci 52:707–719. https://doi.org/10.2135/cropsci2011.06.0299

Caverzan A, Casassola A, Brammer SP (2016) Antioxidant responses of wheat plants under stress. Genet Mol Biol 39:1–6. https://doi.org/10.1590/1678-4685-GMB-2015-0109

Cericola F, Jahoor A, Orabi J et al (2017) Optimizing training population size and genotyping strategy for genomic prediction using association study results and pedigree information. A case of study in advanced wheat breeding lines. PLoS One 12. https://doi.org/10.1371/journal.pone.0169606

Cheong BE, Ho WWH, Biddulph B et al (2019) Phenotyping reproductive stage chilling and frost tolerance in wheat using targeted metabolome and lipidome profiling. Metabolomics 15:144. https://doi.org/10.1007/s11306-019-1606-2

Cheong BE, Onyemaobi O, Wing Ho Ho W et al (2020) Phenotyping the chilling and freezing responses of young microspore stage wheat spikes using targeted metabolome and lipidome profiling. Cells 9(5):1309. https://doi.org/10.3390/cells9051309

Chopra RK, Shukla S, Singh K et al (2012) Characterization of high yielding and drought tolerant RILs identified from wheat cross WL711 x C306 RIL mapping population using drought susceptibility index (DSI) as selection criteria. Indian J Plant Genet Resour 26(1):25–31

Cobb JN, Juma RU, Biswas PS et al (2019) Enhancing the rate of genetic gain in public-sector plant breeding programs: lessons from the breeder's equation. Theor Appl Genet 132:627–645. https://doi.org/10.1007/s00122-019-03317-0

Cossani CM, Reynolds MP (2012) Physiological traits for improving heat tolerance in wheat. Plant Physiol 160:1710–1718. https://doi.org/10.1104/pp.112.207753

Crain J, Mondal S, Rutkoski J et al (2018a) Combining high-throughput phenotyping and genomic information to increase prediction and selection accuracy in wheat breeding. Plant Genome 11:170043. https://doi.org/10.3835/plantgenome2017.05.0043

Crain J, Mondal S, Rutkoski J et al (2018b) Combining high-throughput phenotyping and genomic information to increase prediction and selection accuracy in wheat breeding. Plant Genome 11:1–14. https://doi.org/10.3835/plantgenome2017.05.0043

Crespo-Herrera LA, Crossa J, Huerta-Espino J et al (2017) Genetic yield gains in CIMMYT'S international elite spring wheat yield trials by modeling the genotype × environment interaction. Crop Sci 57:789. https://doi.org/10.2135/cropsci2016.06.0553

Crespo-Herrera LA, Crossa J, Huerta-Espino J et al (2018) Genetic gains for grain yield in cimmyt's semi-arid wheat yield trials grown in suboptimal environments. Crop Sci 58:1890–1898. https://doi.org/10.2135/cropsci2018.01.0017

Crossa J, De Los Campos G, Pérez P et al (2010) Prediction of genetic values of quantitative traits in plant breeding using pedigree and molecular markers. Genetics. https://doi.org/10.1534/genetics.110.118521

Crossa J, Vargas M, Cossani CM et al (2015) Evaluation and interpretation of interactions. Agron J 107:736–747. https://doi.org/10.2134/agronj2012.0491

Crossa J, Jarquín D, Franco J et al (2016) Genomic prediction of gene bank wheat landraces. G3 Genes Genomes Genet 6. https://doi.org/10.1534/g3.116.029637

Crossa J, Martini JWR, Gianola D et al (2019) Deep kernel and deep learning for genome-based prediction of single traits in multienvironment breeding trials. Front Genet 10:1–13. https://doi.org/10.3389/fgene.2019.01168

Czyczyło-Mysza I, Marcińska I, Skrzypek E et al (2014) QTL mapping for germination of seeds obtained from previous wheat generation under drought. Cent Eur J Biol 9:374. https://doi.org/10.2478/s11535-013-0273-y

Dar EA, Brar AS, Singh KB (2017) Water use and productivity of drip irrigated wheat under variable climatic and soil moisture regimes in North-West, India. Agric Ecosyst Environ 248:9–19. https://doi.org/10.1016/j.agee.2017.07.019

Daryanto S, Wang L, Jacinthe P-A (2016) Global synthesis of drought effects on maize and wheat production. PLoS ONE 11:1–15. https://doi.org/10.1371/journal.pone.0156362

Dawood MFA, Abeed AHA, Aldaby EES (2019) Titanium dioxide nanoparticles model growth kinetic traits of some wheat cultivars under different water regimes. Plant Physiol Reports 24:129–140. https://doi.org/10.1007/s40502-019-0437-5

de los Campos G, Vazquez AI, Fernando R et al (2013) Prediction of complex human traits using the genomic best linear unbiased predictor. PLoS Genet 9:e1003608. https://doi.org/10.1371/journal.pgen.1003608

Debernardi JM, Tricoli DM, Ercoli MF et al (2020) A GRF–GIF chimeric protein improves the regeneration efficiency of transgenic plants. Nat Biotechnol 38:1274. https://doi.org/10.1038/s41587-020-0703-0

Denčić S, Kastori R, Kobiljski B, Duggan B (2000) Evaluation of grain yield and its components in wheat cultivars and landraces under near optimal and drought conditions. Euphytica 113:43. https://doi.org/10.1023/A:1003997700865

Deng XP, Shan L, Inanaga S, Inoue M (2005) Water-saving approaches for improving wheat production. J Sci Food Agric 85:1379–1388. https://doi.org/10.1002/jsfa.2101

Djanaguiraman M, Boyle DL, Welti R et al (2018) Decreased photosynthetic rate under high temperature in wheat is due to lipid desaturation, oxidation, acylation, and damage of organelles. BMC Plant Biol 18:1–17. https://doi.org/10.1186/s12870-018-1263-z

Dong H, Wang R, Yuan Y et al (2018) Evaluation of the potential for genomic selection to improve spring wheat resistance to Fusarium head blight in the Pacific Northwest. Front Plant Sci 9:911. https://doi.org/10.3389/fpls.2018.00911

Edae EA, Byrne PF, Manmathan H et al (2013) Association mapping and nucleotide sequence variation in five drought tolerance candidate genes in spring wheat. Plant Genome 6:2013. https://doi.org/10.3835/plantgenome2013.04.0010

Edae EA, Byrne PF, Haley SD et al (2014) Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. Theor Appl Genet 127:791–807. https://doi.org/10.1007/s00122-013-2257-8

El Habti A, Fleury D, Jewell N et al (2020) Tolerance of combined drought and heat stress is associated with transpiration maintenance and water soluble carbohydrates in wheat grains. Front Plant Sci 11:1–13. https://doi.org/10.3389/fpls.2020.568693

Enghiad A, Ufer D, Countryman AM, Thilmany DD (2017) An overview of global wheat market fundamentals in an era of climate concerns. Intl J Agron 2017. https://doi.org/10.1155/2017/3931897

Fahad S, Hussain S, Matloob A et al (2015) Phytohormones and plant responses to salinity stress: a review. Plant Growth Regul 75:391–404. https://doi.org/10.1007/s10725-014-0013-y

Fahad S, Bajwa AA, Nazir U et al (2017) Crop production under drought and heat stress: plant responses and management options. Front Plant Sci 8:1–16. https://doi.org/10.3389/fpls.2017.01147

Farooq M, Hussain M, Wahid A, Siddique KHM (2012) Drought stress in plants: an overview. Plant responses to drought stress: from morphological to molecular features. Springer, Berlin, pp 1–33

Farooq M, Hussain M, Siddique KHM (2014) Drought stress in wheat during flowering and grain-filling periods. Crit Rev Plant Sci 33:331–349

Foulkes MJ, Snape JW, Shearman VJ et al (2007) Genetic progress in yield potential in wheat: recent advances and future prospects. J Agric Sci 145:17–29. https://doi.org/10.1017/s0021859607006740

Gahlaut V, Jaiswal V, Tyagi BS et al (2017) QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. PLoS ONE 12:e0182857. https://doi.org/10.1371/journal.pone.0182857

Gaudelli NM, Komor AC, Rees HA et al (2017) Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature 551:464–471. https://doi.org/10.1038/nature24644

Gaudelli NM, Lam DK, Rees HA et al (2020) Directed evolution of adenine base editors with increased activity and therapeutic application. Nat Biotechnol 38:892–900. https://doi.org/10.1038/s41587-020-0491-6

Genc Y, Oldach K, Taylor J, Lyons GH (2016) Uncoupling of sodium and chloride to assist breeding for salinity tolerance in crops. New Phytol 210:145. https://doi.org/10.1111/nph.13757

Genetic Literacy Project: Global gene editing regulation tracker and index. https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/. Accessed 20 Oct 2020

Ghosh S, Watson A, Gonzalez-Navarro OE et al (2018) Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. Nat Protoc 13:2944–2963. https://doi.org/10.1038/s41596-018-0072-z

González-Camacho JM, Ornella L, Pérez-Rodríguez P et al (2018) Applications of machine learning methods to genomic selection in breeding wheat for rust resistance. Plant Genome 11:170104. https://doi.org/10.3835/plantgenome2017.11.0104

Gonzalez-Sanchez A, Frausto-Solis J, Ojeda-Bustamante W (2014) Predictive ability of machine learning methods for massive crop yield prediction. Span J Agric Res 12. https://doi.org/10.5424/sjar/2014122-4439

Guo J, Xu W, Yu X et al (2016) Cuticular wax accumulation is associated with drought tolerance in wheat near-isogenic lines. Front Plant Sci 7:1809. https://doi.org/10.3389/fpls.2016.01809

Gupta P, Balyan H, Gahlaut V (2017) QTL Analysis for drought tolerance in wheat: present status and future possibilities. Agronomy 7:5. https://doi.org/10.3390/agronomy7010005

Habbasha E, Ramadan A (2014) Wheat Production in the arid regions by using drip irrigation system. Intl J Adv Res 2:84–96

Hamada A, Nitta M, Nasuda S et al (2012) Novel QTLs for growth angle of seminal roots in wheat (*Triticum aestivum* L.) 395–405. https://doi.org/10.1007/s11104-011-1075-5

Hammer K, Knüpffer H (2015) Genetic resources of triticum. In: Ogihara Y, Takumi S, Handa H (eds) Advances in wheat genetics: from genome to field. Springer Japan, Tokyo, pp 23–31

Hasheminasab H, Taghi Assad M, Aliakbari A, Rasoul Sahhafi S (2012) Influence of drought stress on oxidative damage and antioxidant defense systems in tolerant and susceptible wheat genotypes. J Agric Sci 4:20–30. https://doi.org/10.5539/jas.v4n8p20

Hassan NU, Mahmood Q, Waseem A et al (2013) Assessment of heavy metals in wheat plants irrigated with contaminated wastewater. Polish J Environ Stud 22

Hazratkulova S, Sharma RC, Alikulov S et al (2012) Analysis of genotypic variation for normalized difference vegetation index and its relationship with grain yield in winter wheat under terminal heat stress. Plant Breed 131:716. https://doi.org/10.1111/pbr.12003

Heffner EL, Jannink J-L, Sorrells ME (2011a) Genomic selection accuracy using multifamily prediction models in a wheat breeding program. Plant Genome J 4. https://doi.org/10.3835/plantgenome.2010.12.0029

Heffner EL, Jannink J-L, Sorrells ME (2011b) Genomic selection accuracy using multifamily prediction models in a wheat breeding program. Plant Genome 4. https://doi.org/10.3835/plantgenome2010.12.0029

Hein NT, Wagner D, Bheemanahalli R et al (2019) Integrating field-based heat tents and cyber-physical system technology to phenotype high night-time temperature impact on winter wheat. Plant Methods 15:1–15. https://doi.org/10.1186/s13007-019-0424-x

Heslot N, Akdemir D, Sorrells ME, Jannink J-L (2014) Integrating environmental covariates and crop modeling into the genomic selection framework to predict genotype by environment interactions. Theor Appl Genet 127:463–480. https://doi.org/10.1007/s00122-013-2231-5

Hoffstetter A, Cabrera A, Huang M, Sneller C (2016) Optimizing training population data and validation of genomic selection for economic traits in soft winter wheat. G3 Genes Genomes Genet 6. https://doi.org/10.1534/g3.116.032532

Hoisington D, Khairallah M, Reeves T et al (1999) Plant genetic resources: what can they contribute toward increased crop productivity? Proc Natl Acad Sci USA 96:5937–5943

Hong MJ, Kim DY, Nam BM et al (2019) Characterization of novel mutants of hexaploid wheat (*Triticum aestivum* L.) with various depths of purple grain color and antioxidant capacity. J Sci Food Agric 99:55–63. https://doi.org/10.1002/jsfa.9141

Hoover DL, Wilcox KR, Young KE (2018) Experimental droughts with rainout shelters: a methodological review. Ecosphere 9. https://doi.org/10.1002/ecs2.2088

Hu P, Zheng Q, Luo Q et al (2020) Genome-wide association study of yield and related traits in common wheat under salt-stress conditions 1–34. https://doi.org/10.21203/rs.3.rs-22191/v1

Iqbal MJ (2018) Role of osmolytes and antioxidant enzymes for drought tolerance in wheat. InTech. http://dx.doi.org/10.5772/intechopen.75926

Jarquín D, Crossa J, Lacaze X et al (2014) A reaction norm model for genomic selection using high-dimensional genomic and environmental data. Theor Appl Genet. https://doi.org/10.1007/s00122-013-2243-1

Jinek M, Chylinski K, Fonfara I et al (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (80)337:816–821. https://doi.org/10.1126/science.1225829

Joukhadar R, Daetwyler HD, Bansal UK et al (2017) Genetic diversity, population structure and ancestral origin of Australian wheat. Front Plant Sci 8:1–15. https://doi.org/10.3389/fpls.2017.02115

Juliana P, Singh RP, Singh PK et al (2017) Comparison of models and whole-genomeprofiling approaches for genomic-enabled prediction of Septoria tritici blotch, *Stagonospora nodorum* blotch, and Tan spot resistance in wheat. Plant Genome 10. https://doi.org/10.3835/plantgenome2016.08.0082

Juliana P, Montesinos-López OA, Crossa J et al (2019a) Integrating genomic-enabled prediction and high-throughput phenotyping in breeding for climate-resilient bread wheat. Theor Appl Genet 132:177. https://doi.org/10.1007/s00122-018-3206-3

Juliana P, Poland J, Huerta-Espino J et al (2019b) Improving grain yield, stress resilience and quality of bread wheat using large-scale genomics. Nat Genet 51:1530–1539. https://doi.org/10.1038/s41588-019-0496-6

Kadioglu A, Terzi R, Saruhan N, Saglam A (2012) Current advances in the investigation of leaf rolling caused by biotic and abiotic stress factors. Plant Sci 182:42–48. https://doi.org/10.1016/j.plantsci.2011.01.013

Kaur R, Bhardwaj R, Sharma R et al (2016) Hormonal regulation of drought stress in plants. Water Stress and Crop Plants A Sustain Approach. Wiley, Chichester, UK, pp 582–599

Kaya C, Tuna AL, Yokaş I (2009) The role of plant hormones in plants under salinity stress 45–50. https://doi.org/10.1007/978-1-4020-9065-3_5

Khadka RB, Raut P (2011) Title : system of wheat intensification (SWI): A new concept on low input technology for increasing wheat yield in marginal land. Authors: Ram Bahadur Khadka Program Coordinator, European Union Food Facility Project Forum for Awareness and Youth Acti

Kim D, Alptekin B, Budak H (2018) CRISPR/Cas9 genome editing in wheat. Funct Integr Genom 18:31–41. https://doi.org/10.1007/s10142-017-0572-x

Kristensen PS, Jahoor A, Andersen JR et al (2018) Genome-wide association studies and comparison of models and cross-validation strategies for genomic prediction of quality traits in advanced winter wheat breeding lines. Front Plant Sci 9:69. https://doi.org/10.3389/fpls.2018.00069

Kumar M, Hasan M, Arora A et al (2015) Sodium chloride-induced spatial and temporal manifestation in membrane stability index and protein profiles of contrasting wheat (*Triticum aestivum* L.) genotypes under salt stress. Indian J Plant Physiol 20. https://doi.org/10.1007/s40502-015-0157-4

La Russa MF, Qi LS (2015) The new state of the art: CRISPR for gene activation and repression. Mol Cell Biol MCB.00512-15. https://doi.org/10.1128/MCB.00512-15

Ladejobi O, Mackay IJ, Poland J et al (2019) Reference genome anchoring of high-density markers for association mapping and genomic prediction in European winter wheat. Front Plant Sci 10:1278. https://doi.org/10.3389/fpls.2019.01278

Laxa M, Liebthal M, Telman W et al (2019) The role of the plant antioxidant system in drought tolerance. Antioxidants 8:94. https://doi.org/10.3390/antiox8040094

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 871. https://doi.org/10.3389/fpls.2018.01728

Li Y, Liang X, Huang Q et al (2019) Inhibition of Cd accumulation in grains of wheat and rice under rotation mode using composite silicate amendment. RSC Adv 9:35539. https://doi.org/10.1039/c9ra07137g

Liang Z, Chen K, Li T et al (2017) Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nat Commun 8:6–10. https://doi.org/10.1038/ncomms14261

Liu B, Asseng S, Müller C et al (2016) Similar estimates of temperature impacts on global wheat yield by three independent methods. Nat Clim Chang 6:1130–1137. https://doi.org/10.1038/nclimate3115

Liu C, Pinto F, Cossani CM et al (2019) Spectral reflectance indices as proxies for yield potential and heat stress tolerance in spring wheat: Heritability estimates and marker-trait associations. Front Agric Sci Eng 6:296–308. https://doi.org/10.15302/J-FASE-2019269

Lobell DB, Sibley A, Ivan Ortiz-Monasterio J (2012) Extreme heat effects on wheat senescence in India. Nat Clim Chang 2:186–189. https://doi.org/10.1038/nclimate1356

Lonbani M, Arzani A (2011) Morpho-physiological traits associated with terminal drought-stress tolerance in triticale and wheat. Agron Res 9:315–329

Lopez-Cruz M, Crossa J, Bonnett D et al (2015) Increased prediction accuracy in wheat breeding trials using a marker x environment interaction genomic selection model. G3 Genes Genomes Genet Genes|Genomes|Genetics 569–582. https://doi.org/10.1534/g3.114.016097

Loutfy N, El-Tayeb MA, Hassanen AM et al (2012) Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum*). J Plant Res 125:173–184. https://doi.org/10.1007/s10265-011-0419-9

Lu Y, Ye X, Guo R et al (2017) Genome-wide targeted mutagenesis in rice using the CRISPR/Cas9 system. Mol Plant 10:1242–1245. https://doi.org/10.1016/j.molp.2017.06.007

Ma X, Zhang Q, Zhu Q et al (2015) A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. Mol Plant 8:1274–1284. https://doi.org/10.1016/j.molp.2015.04.007

Maes B, Trethowan RM, Reynolds MP et al (2001) The influence of glume pubescence on spikelet temperature of wheat under freezing conditions. Aust J Plant Physiol 28:141–148. https://doi.org/10.1071/pp00049

Marcussen T, Sandve SR, Heier L et al (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 345:1250092. https://doi.org/10.1126/science.1251788

Mason RE, Mondal S, Beecher FW, et al (2010a) QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress. Euphytica 174. https://doi.org/10.1007/s10681-010-0151-x

Mason RE, Mondal S, Beecher FW, et al (2010b) QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress. Euphytica 174. https://doi.org/10.1007/s10681-010-0151-x

Mason RE, Hays DB, Mondal S et al (2013) QTL for yield, yield components and canopy temperature depression in wheat under late sown field conditions. Euphytica 194:243. https://doi.org/10.1007/s10681-013-0951-x

Maulana F, Ayalew H, Anderson JD et al (2018) Genome-wide association mapping of seedling heat tolerance in winter wheat. Front Plant Sci 9:1272. https://doi.org/10.3389/fpls.2018.01272

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829. https://doi.org/11290733

Miransari M, Smith D (2019) Sustainable wheat (*Triticum aestivum* L.) production in saline fields: a review. Crit Rev Biotechnol 39:999–1014. https://doi.org/10.1080/07388551.2019.1654973

Mishra R, Joshi RK (2020) Base editing in crops : current advances, limitations and future implications 20–31. https://doi.org/10.1111/pbi.13225

Mishra A, Singh A, Sharma M et al (2016) Development of EMS-induced mutation population for amylose and resistant starch variation in bread wheat (*Triticum aestivum*) and identification of candidate genes responsible for amylose variation. BMC Plant Biol 16:1–15. https://doi.org/10.1186/s12870-016-0896-z

Mohammady S, Aminian R, Hoshmand S, Khodombashi M (2012) Genomic analysis of carbon isotope discrimination, photosynthesis rate, stomatal conductance, and grain yield in wheat (*Triticum aestivum* L.) under water-stressed conditions. Crop Pasture Sci 63. https://doi.org/10.1071/CP12050

Molero G, Joynson R, Pinera-Chavez FJ et al (2019) Elucidating the genetic basis of biomass accumulation and radiation use efficiency in spring wheat and its role in yield potential. Plant Biotechnol J 1–13:1276. https://doi.org/10.1111/pbi.13052

Mondal S, Mason RE, Huggins T, Hays DB (2015a) QTL on wheat (Triticum aestivum L.) chromosomes 1B, 3D and 5A are associated with constitutive production of leaf cuticular wax and may contribute to lower leaf temperatures under heat stress. Euphytica 201. https://doi.org/10.1007/s10681-014-1193-2

Mondal S, Mason RE, Huggins T, Hays DB (2015b) QTL on wheat (*Triticum aestivum* L.) chromosomes 1B, 3D and 5A are associated with constitutive production of leaf cuticular wax and may contribute to lower leaf temperatures under heat stress. Euphytica 201. https://doi.org/10.1007/s10681-014-1193-2

Mondal S, Rutkoski JE, Velu G et al (2016a) Harnessing diversity in wheat to enhance grain yield, climate resilience, disease and insect pest resistance and nutrition through conventional and modern breeding approaches. Front Plant Sci 7:1–15. https://doi.org/10.3389/fpls.2016.00991

Mondal S, Singh RP, Mason ER et al (2016b) Grain yield, adaptation and progress in breeding for early-maturing and heat-tolerant wheat lines in South Asia. Field Crop Res 192:78. https://doi.org/10.1016/j.fcr.2016.04.017

Mondal S, Dutta S, Crespo-Herrera L et al (2020) Fifty years of semi-dwarf spring wheat breeding at CIMMYT: grain yield progress in optimum, drought and heat stress environments. Field Crop Res 250:107757. https://doi.org/10.1016/j.fcr.2020.107757

Morgounov A, Abugalieva A, Akan K et al (2017) High-yielding winter synthetic hexaploid wheats resistant to multiple diseases and pests. Plant Genet Resour 1–6:273. https://doi.org/10.1017/S147926211700017X

Mujeeb-Kazi A, Rosas V, Roldan S (1996) Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. s.lat. x T. tauschii; 2n = 6x = 42, AABBDD) and its potential utilization for wheat improvement. Genet Resour Crop Evol 43:129–134. https://doi.org/10.1007/BF00126756

Muleta KT, Bulli P, Zhang Z et al (2017) Unlocking diversity in germplasm collections via genomic selection: a case study based on quantitative adult plant resistance to stripe rust in spring wheat. Plant Genome 10:2016. https://doi.org/10.3835/plantgenome2016.12.0124

Mwadzingeni L, Figlan S, Shimelis H et al (2017) Genetic resources and breeding methodologies for improving drought tolerance in wheat. J Crop Improv 31:648. https://doi.org/10.1080/15427528.2017.1345816

Nakaya A, Isobe SN (2012) Will genomic selection be a practical method for plant breeding? Ann Bot 110(6):1303–1316

Naz AA, Dadshani S, Ballvora A et al (2019) Genetic analysis and transfer of favorable exotic QTL alleles for grain yield across D genome using two advanced backcross wheat populations. Front Plant Sci 10:1–9. https://doi.org/10.3389/fpls.2019.00711

Nuttall JG, Perry EM, Delahunty AJ et al (2019) Frost response in wheat and early detection using proximal sensors. J Agron Crop Sci 205:220–234. https://doi.org/10.1111/jac.12319

Odilbekov F, Armonienė R, Koc A et al (2019) GWAS-assisted genomic prediction to predict resistance to Septoria tritici blotch in Nordic winter wheat at seedling stage. Front Genet 10:1224. https://doi.org/10.3389/fgene.2019.01224

Ogbonnaya FC, Abdalla O, Mujeeb-Kazi A et al (2013) Synthetic hexaploids: harnessing species of the primary gene pool for wheat improvement. Plant Breed Rev 37:35–122. https://doi.org/10.1002/9781118497869.ch2

ONU (2019) World population prospects 2019

Ornella L, González-Camacho JM, Dreisigacker S, Crossa J (2017) Applications of genomic selection in breeding wheat for rust resistance. Methods Mol Biol 1659:173–182. https://doi.org/10.1007/978-1-4939-7249-4_15

Oyiga BC, Sharma RC, Baum M et al (2018) Allelic variations and differential expressions detected at quantitative trait loci for salt stress tolerance in wheat. Plant Cell Environ 41:919. https://doi.org/10.1111/pce.12898

Paliwal R, Röder MS, Kumar U et al (2012) QTL mapping of terminal heat tolerance in hexaploid wheat (*T. aestivum* L.). Theor Appl Genet 125:561–575. https://doi.org/10.1007/s00122-012-1853-3

Pantazi XE, Moshou D, Alexandridis T et al (2016) Wheat yield prediction using machine learning and advanced sensing techniques. Comput Electron Agric 121:57–65. https://doi.org/10.1016/j.compag.2015.11.018

Pérez P, de los Campos G (2014) Genome-wide regression and prediction with the BGLR statistical package. Genetics 198:483–495. https://doi.org/10.1534/genetics.114.164442

Pérez-Rodríguez P, Crossa J, Rutkoski J et al (2017) Single-step genomic and pedigree genotype × environment interaction models for predicting wheat lines in international environments. Plant Genome 10(2). https://doi.org/10.3835/plantgenome2016.09.0089

Pestsova E, Röder M (2002) Microsatellite analysis of wheat chromosome 2D allows the reconstruction of chromosomal inheritance in pedigrees of breeding programmes. Theor Appl Genet 106:84. https://doi.org/10.1007/s00122-002-0998-x

Pinto RS, Reynolds MP, Mathews KL et al (2010) Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theor Appl Genet 121:1001–1021. https://doi.org/10.1007/s00122-010-1351-4

Poland J, Endelman J, Dawson J et al (2012) Genomic selection in wheat breeding using genotyping-by-sequencing. Plant Genome 5. https://doi.org/10.3835/plantgenome2012.06.0006

Pradhan GP, Prasad PVV, Fritz AK et al (2012) Effects of drought and high temperature stress on synthetic hexaploid wheat. Funct Plant Biol 39:190–198. https://doi.org/10.1071/FP11245

Qaseem MF, Qureshi R, Muqaddasi QH et al (2018) Genome-wide association mapping in bread wheat subjected to independent and combined high temperature and drought stress. PLoS ONE 13:e0199121. https://doi.org/10.1371/journal.pone.0199121

Rawson HM, Macpherson HG (2000) Irrigated wheat : managing your crop. Food and Agriculture Organization of the United Nations, Rome, Italy

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS ONE 8: https://doi.org/10.1371/journal.pone.0066428

Rebetzke GJ, Condon AG, Farquhar GD et al (2008) Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. Theor Appl Genet 118:123. https://doi.org/10.1007/s00122-008-0882-4

Reif JC, Zhang P, Dreisigacker S et al (2005) Wheat genetic diversity trends during domestication and breeding. Theor Appl Genet 110:859–864. https://doi.org/10.1007/s00122-004-1881-8

Ren S, Qin Q, Ren H et al (2019) Heat and drought stress advanced global wheat harvest timing from 1981–2014. Remote Sens 11:971. https://doi.org/10.3390/rs11080912

Reynolds MP, Langridge P (2016) Physiological breeding. Curr Opin Plant Biol 31:162–171. https://doi.org/10.1016/j.pbi.2016.04.005

Reynolds MP, Pierre C Saint, Saad ASI et al (2007) Evaluating potential genetic gains in wheat associated with stress-adaptive trait expression in elite genetic resources under drought and geat Stress. Crop Sci 47:S-172. https://doi.org/10.2135/cropsci2007.10.0022IPBS

Reynolds MP, Pask AJD, Hoppitt WJE et al (2017) Strategic crossing of biomass and harvest index—source and sink—achieves genetic gains in wheat. Euphytica 213:1. https://doi.org/10.1007/s10681-017-2040-z

Roca Paixão JF, Gillet F-X, Ribeiro TP et al (2019) Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a histone acetyltransferase. Sci Rep 9:8080. https://doi.org/10.1038/s41598-019-44571-y

Röder MS, Huang XQ, Börner A (2008) Fine mapping of the region on wheat chromosome 7D controlling grain weight. Funct Integr Genom 8:79–86. https://doi.org/10.1007/s10142-007-0053-8

Rosyara U, Kishii M, Payne T et al (2019) Genetic contribution of synthetic hexaploid wheat to CIMMYT's spring bread wheat breeding germplasm. Sci Rep 9:1–11. https://doi.org/10.1038/s41598-019-47936-5

Royo A, Abió D (2003) Salt tolerance in durum wheat cultivars. Span J Agric Res 1:27. https://doi.org/10.5424/sjar/2003013-32

Ruelland E, Zachowski A (2010) How plants sense temperature. Environ Exp Bot 69

Ruqiang X, Qixin S, Shuzhen Z (1996) Chromosomal location of genes for heat tolerance as measured by membrane thermostability of common wheat cv. Hope. Yi Chuan = Hereditas 18 (4):1–3

Rutkoski JE, Poland JA, Singh RP et al (2015) Genomic selection for quantitative adult plant stem rust resistance in wheat. Plant Genome 7:0. https://doi.org/10.3835/plantgenome2014.02.0006

Rutkoski J, Poland J, Mondal S et al (2016) Canopy temperature and vegetation indices from high-throughput phenotyping improve accuracy of pedigree and genomic selection for grain yield in wheat. G3 Genes Genomes Genet 6. https://doi.org/10.1534/g3.116.032888

Saint Pierre C, Burgueño J, Crossa J et al (2016) Genomic prediction models for grain yield of spring bread wheat in diverse agro-ecological zones. Sci Rep 6:1. https://doi.org/10.1038/srep27312

Sallam A, Amro A, EL-Akhdar A et al (2018) Genetic diversity and genetic variation in morpho-physiological traits to improve heat tolerance in spring barley. Mol Biol Rep 45:2441. https://doi.org/10.1007/s11033-018-4410-6

Sallam A, Alqudah AM, Dawood MFA et al (2019) Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetics research. Intl J Mol Sci 20:3137. https://doi.org/10.3390/ijms20133137

Sansaloni C, Franco J, Santos B et al (2020) Diversity analysis of 80,000 wheat accessions reveals consequences and opportunities of selection footprints. Nat Commun 11:4572. https://doi.org/10.1038/s41467-020-18404-w

Sarinelli JM, Murphy JP, Tyagi P et al (2019) Training population selection and use of fixed effects to optimize genomic predictions in a historical USA winter wheat panel. Theor Appl Genet 132:1247. https://doi.org/10.1007/s00122-019-03276-6

Sattar A, Sher A, Ijaz M et al (2020) Terminal drought and heat stress alter physiological and biochemical attributes in flag leaf of bread wheat. PLoS ONE 15:e0232974. https://doi.org/10.1371/journal.pone.0232974

Savicka M, Škute N (2010) Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.) Aukštos temperatūros poveikis kviečių (*Triticum aestivum* L.). Ekologija 56:26–33. https://doi.org/10.2478/v10055-010-0004-x

Schmidt J, Claussen J, Wörlein N et al (2020) Drought and heat stress tolerance screening in wheat using computed tomography. Plant Methods 16:1–12. https://doi.org/10.1186/s13007-020-00565-w

Sehgal D, Autrique E, Singh R et al (2017) Identification of genomic regions for grain yield and yield stability and their epistatic interactions. Sci Rep 7:41578. https://doi.org/10.1038/srep41578

Sehgal D, Rosyara U, Mondal S et al (2020) Incorporating genome-wide association mapping results into genomic prediction models for grain yield and yield stability in CIMMYT spring bread wheat. Front Plant Sci 11:197. https://doi.org/10.3389/fpls.2020.00197

Semenov MA, Halford NG (2009) Identifying target traits and molecular mechanisms for wheat breeding under a changing climate. J Exp Bot 60(10):2791–2804

Shah NH, Paulsen GM (2003) Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant Soil 257:219–226. https://doi.org/10.1023/A:1026237816578

Sharma R, Morgounov A, Braun H et al (2012) Yield stability analysis of winter wheat genotypes targeted to semi-arid environments in the international winter wheat improvement program. Intl J Plant Breed 6:7–13

Sharma DK, Torp AM, Rosenqvist E et al (2017) QTLs and potential candidate genes for heat stress tolerance identified from the mapping populations specifically segregating for Fv/Fm in wheat. Front Plant Sci 8:1668. https://doi.org/10.3389/fpls.2017.01668

Sharma A, Shahzad B, Kumar V et al (2019) Phytohormones regulate accumulation of osmolytes under abiotic stress. Biomolecules 9:285. https://doi.org/10.3390/biom9070285

Shaul O (2015) Unique aspects of plant nonsense-mediated mRNA decay. Trends Plant Sci 20:767–779. https://doi.org/10.1016/j.tplants.2015.08.011

Shi J, Gao H, Wang H et al (2017) ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207–216. https://doi.org/10.1111/pbi.12603

Simeonov V, Stratis JA, Samara C et al (2003) Assessment of the surface water quality in Northern Greece. Water Res 37:4119. https://doi.org/10.1016/S0043-1354(03)00398-1

Singh RK, Mishra B, Chauhan MS et al (2002) Solution culture for screening rice varieties for sodicity tolerance. J Agric Sci 139:327. https://doi.org/10.1017/S0021859602002447

Singh D, Wang X, Kumar U et al (2019a) High-throughput phenotyping enabled genetic dissection of crop lodging in wheat. Front Plant Sci 10:394. https://doi.org/10.3389/fpls.2019.00394

Singh N, Wu S, Raupp WJ et al (2019b) Efficient curation of genebanks using next generation sequencing reveals substantial duplication of germplasm accessions. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-018-37269-0

Skinner DZ, Garland-Campbell KA (2008) The relationship of LT50 to prolonged freezing survival in winter wheat. Can J Plant Sci 88:885–889. https://doi.org/10.4141/CJPS08007

Snyder RL, Melo-Abreu JP, Matulich S (2005). Frost Protection: fundamentals, practice and economics volume 1 and 2. FAO Environ Nat Res Service Series, No. 10—FAO, Rome

Soriano JM, Alvaro F (2019) Discovering consensus genomic regions in wheat for root-related traits by QTL meta-analysis. Sci Rep 9:1–4. https://doi.org/10.1038/s41598-019-47038-2

Sreenivasulu N, Sopory SK, Kavi Kishor PB (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. Gene 388:1–13. https://doi.org/10.1016/j.gene.2006.10.009

Sukumaran S, Yu J (2014) Association mapping of genetic resources: Achievements and future perspectives. In: Tuberosa R, Graner A, Frison E (eds) Genomics of plant genetic resources: volume 1. Managing, sequencing and mining genetic resources. https://doi.org/10.1007/978-94-007-7572-5_9. Springer, New York

Sukumaran S, Reynolds MP, Lopes MS, Crossa J (2015) Genome-wide association study for adaptation to agronomic plant density: a component of high yield potential in spring wheat. Crop Sci 55:2609. https://doi.org/10.2135/cropsci2015.03.0139

Sun QX, Quick JS (1991) Chromosomal locations of genes for heat tolerance in tetraploid wheat. Cereal Res Commun 19(4):431–437

Talukder SK, Babar MA, Vijayalakshmi K et al (2014) Mapping QTL for the traits associated with heat tolerance in wheat (*Triticum aestivum* L.). BMC Genet 15:97. https://doi.org/10.1186/s12863-014-0097-4

Tattaris M, Reynolds MP, Chapman SC (2016) A direct comparison of remote sensing approaches for high-throughput phenotyping in plant breeding. Front Plant Sci 7:1131. https://doi.org/10.3389/fpls.2016.01131

Thomas H, Ougham H (2014) The stay-green trait. J Exp Bot 65(14):3889–3900. https://doi.org/10.1093/jxb/eru037 Epub 2014 Mar 5

Tiwari C, Wallwork H, Kumar U et al (2013) Molecular mapping of high temperature tolerance in bread wheat adapted to the Eastern Gangetic Plain region of India. Field Crop Res 154:201. https://doi.org/10.1016/j.fcr.2013.08.004

Trnka M, Feng S, Semenov MA et al (2019) Mitigation efforts will not fully alleviate the increase in water scarcity occurrence probability in wheat-producing areas. Sci Adv 5:eaau2406. https://doi.org/10.1126/sciadv.aau2406

Tshikunde NM, Mashilo J, Shimelis H, Odindo A (2019) Agronomic and physiological traits, and associated quantitative trait loci (QTL) affecting yield response in wheat (*Triticum aestivum* L.): a review. Front Plant Sci 10

United Nations, Department of Economic and Social Affairs, Population Division (2019). World population prospects 2019: Highlights (ST/ESA/SER.A/423)

VanGinkel M, Ortiz R (2018) Cross the best with the best, and select the best: HELP in breeding selfing crops. Crop Sci 58:17–30. https://doi.org/10.2135/cropsci2017.05.0270

Verma V, Foulkes MJ, Worland AJ et al (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135:255. https://doi.org/10.1023/B:EUPH.0000013255.31618.14

Véry A-A, Sentenac H (2003) Molecular mechanisms and regulation of K + transport in higher plants. Annu Rev Plant Biol 54:575–603. https://doi.org/10.1146/annurev.arplant.54.031902.134831

Vijayalakshmi K, Fritz AK, Paulsen GM et al (2010) Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Mol Breed 26:163. https://doi.org/10.1007/s11032-009-9366-8

Villareal RL, Sayre K, Banuelos O, Mujeeb-Kazi A (2001) Registration of four synthetic hexaploid wheat (*Triticum turgidum/Aegilops tauschii*) germplasm lines tolerant to waterlogging. Crop Sci 41:274. https://doi.org/10.2135/cropsci2001.411274x

Voss-Fels KP, Robinson H, Mudge SR et al (2018) VERNALIZATION1 modulates root system architecture in wheat and barley. Mol Plant 11:226–229. https://doi.org/10.1016/j.molp.2017.10.005

Wang Y, Zhang X, Liu X et al (2013) The effects of nitrogen supply and water regime on instantaneous WUE, time-integrated WUE and carbon isotope discrimination in winter wheat. Field Crop Res 144:236–244. https://doi.org/10.1016/j.fcr.2013.01.021

Wang Y, Cheng X, Shan Q et al (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32:947–951. https://doi.org/10.1038/nbt.2969

Wang JY, Xiong YC, Li FM et al (2017) Effects of drought stress on morphophysiological traits, biochemical characteristics, yield, and yield components in different ploidy wheat: a meta-analysis. In: Advances in agronomy. Academic Press Inc., pp 139–173

Wardlaw IF, Willenbrink J (2000) Mobilization of fructan reserves and changes in enzyme activities in wheat stems correlate with water stress during kernel filling. New Phytol 148:413–422. https://doi.org/10.1046/j.1469-8137.2000.00777.x

Watson A, Ghosh S, Williams M, et al (2017) Speed breeding: a powerful tool to accelerate crop research and breeding. bioRxiv 1–17. https://doi.org/10.1101/161182

Watson A, Ghosh S, Williams MJ et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4:23–29. https://doi.org/10.1038/s41477-017-0083-8

Xie K, Minkenberg B, Yang Y (2015) Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. Proc Natl Acad Sci USA 112:3570–3575. https://doi.org/10.1073/pnas.1420294112

Xu W, Jia L, Shi W et al (2013) Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. New Phytol 197:139–150. https://doi.org/10.1111/nph.12004

Yang J, Sears RG, Gill BS, Paulsen GM (2002) Growth and senescence characteristics associated with tolerance of wheat-alien amphiploids to high temperature under controlled conditions. Euphytica 126:185–193. https://doi.org/10.1023/A:1016365728633

Yin X, Biswal AK, Dionora J et al (2017) CRISPR-Cas9 and CRISPR-Cpf1 mediated targeting of a stomatal developmental gene EPFL9 in rice. Plant Cell Rep 36:745–757. https://doi.org/10.1007/s00299-017-2118-z

Yu X, Li X, Guo T et al (2016) Genomic prediction contributing to a promising global strategy to turbocharge gene banks. Nat Plants 2:16150. https://doi.org/10.1038/nplants.2016.150

Yu S, Wu J, Wang M et al (2020) Haplotype variations in QTL for salt tolerance in Chinese wheat accessions identified by marker-based and pedigree-based kinship analyses. Crop J. https://doi.org/10.1016/j.cj.2020.03.007

Zhang H, Cui F, Wang L et al (2013) Conditional and unconditional QTL mapping of drought-tolerance-related traits of wheat seedling using two related RIL populations. J Genet 92:213. https://doi.org/10.1007/s12041-013-0253-z

Zhao C, Liu B, Piao S et al (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci USA 201701762:9326. https://doi.org/10.1073/pnas.1701762114

Chapter 3
Resistance to Abiotic Stress: Theory and Applications in Maize Breeding

R. N. Gadag, Jayant S. Bhat, Ganapati Mukri, G. K. Chikkappa, Ramesh Kumar, Sarita Yadav, Pranjal Yadava, M. L. Nithyashree, Gopalakrishna K. Naidu, Seema Sheoran, and Sunil Kumar Yadav

Abstract Maize, being a widely adapted crop, cultivated across the globe, is supporting both livelihood security and food security of mankind. Cultivated maize is more prone to abiotic stresses as compared to their wild counterpart. Natural mutation followed by natural and artificial selections contributed to yield gains in maize. However, due to the hindrance posed by the abiotic stresses, genetic potential of maize could not be realized to the fullest. Many plant breeding interventions were made to utilize the available genetic resources to breed for stress resilient maize.

R. N. Gadag · G. Mukri (✉)
Division of Genetics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi 110012, India
e-mail: ganapati.mukri@icar.gov.in

J. S. Bhat
Regional Research Centre (ICAR-Indian Agricultural Research Institute), Dharwad, Karnataka 580005, India

G. K. Chikkappa
ICAR-India Institute of Maize Research, PUSA Campus, Unit Office, New Delhi 110012, India

R. Kumar · S. Sheoran
ICAR-India Institute of Maize Research, PAU Campus, Ludhiana 141004, India

S. Yadav
ICAR-National Institute for Plant Biotechnology, PUSA Campus, New Delhi 110012, India

P. Yadava
Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi 110012, India

M. L. Nithyashree
Division of Agricultural Economics, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi 110012, India

G. K. Naidu
All India Coordinated Research Project on Maize, University of Agricultural Sciences, Dharwad 580005, India

S. K. Yadav
Department of Biophysics, University of Delhi South Campus, New Delhi 110021, India

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However, efforts made to breed for high yielding maize through traditional plant breeding approaches and improvement achieved are not matching with the demands. To understand the target traits, geneticist and breeders dissected the traits using modern tools and techniques. Genetic mapping of yield and yield component traits under abiotic stresses opened the new way of marker assisted maize breeding for stress tolerance. Development of different trait specific breeding populations supports the novel findings. Fine mapping and positional cloning experiments provided clear understanding of complex traits like abiotic stresses. The approaches like forward breeding using molecular markers and genomic selection are increasingly becoming cost effective new tools for breeders to accelerate the process of new cultivar development with increased tolerance to abiotic stresses. Genetic variations not present in the wild or cultivated species of maize are also utilized in genetic improvement of maize by transgenic approaches. This has resulted in effective utilization of cross boundary information across different and /or unrelated species. Though the technique is robust, considering societal taboos, this approach has been given less encouragement. Alternate to this, genome editing approach played a remarkable role in improving target traits in maize. The modification in *ARGOS* gene is one of the landmark achievements in drought tolerance in maize. The specificity of genetic alteration followed by gain in genetic improvement will further be enhanced by base editing techniques. Maize being a well-studied model plant and various breeding concepts have been derived by understanding the genetics of traits; application of novel breeding approaches will certainly favor breeding for stress tolerance.

Keywords Abiotic stresses · Genetic resources · Genetic approaches · Molecular breeding · Transgenics · Bioinformatic tools · Genome editing

3.1 Introduction

Maize is cultivated widely throughout the world and is one of the top three cereal crops globally. It is grown on an area of 193.7 mha with a production of 1147.6 mt and productivity of 5.9 tha^{-1} (FAOSTAT 2018). Global maize production has now surpassed rice and wheat and the annual rate of increase in its production is twice that of rice and thrice that of wheat (Fischer et al. 2014). The increase in maize area and production, among cereals, was highest during the period 2006–2015 and is expected to keep the pace during 2016–2025 (OECD/FAO 2016). During this period (2006–2015), the increase in productivity through the deployment of improved varieties also contributed to gains in maize production. There is also an unprecedented increase in demand for maize which increased at a faster rate than that for rice or wheat. Besides being consumed directly by humans, maize is also used as animal feed, poultry feed and as industrial raw material for corn ethanol, corn starch, corn oil and corn syrup. With the 45% increase in demand for maize

(compared to 30% for wheat and 32% for rice), it is the number one crop in the estimated global demand for cereals (IFPRI 2003).

Maize plant is subjected to an array of environmental variations and stresses throughout its growth period. This has been exacerbated by the effects of climate change and degradation of environment due to non-judicious human activities. Thus, the stresses caused by physical environment, such as drought, salinity, waterlogging, cold, nutrient deficiency and mineral toxicity are major hurdles to global food security and are responsible for substantial maize yield losses. The agricultural production including maize production is affected much by climate change due to its dependence on prevailing weather conditions. The modeling studies on climate change have predicted substantial increase in earth's temperature in the future (IPCC 2007), which could lead to heavy loss in maize production, especially in the tropical regions (Cairns et al. 2012). Maize is grown mostly as rainfed crop on marginal environments, making it prone to variety of stresses caused by biotic and abiotic factors. The major abiotic factors affecting maize are drought, waterlogging, nutrient-stress, high temperature and salinity.

The global temperatures are expected to increase and patterns of rainfall are predicted to change substantially as a result of the phenomenon of climate change. This could lead to loss in crop productivity. The abiotic stresses like salinity and drought, affect the maize crop in semi-arid tropical regions. Drought and heat stresses usually co-occur and are the two most important abiotic stresses (Tandzi et al. 2019). Yield losses due to abiotic stresses during flowering and the early grain-filling of maize kernels can reduce the grain yield by 46–90% (Grzesiak et al. 1999; Cakir 2004).

Major abiotic stresses affecting maize growth and productivity worldwide are drought, salt stress, heat and cold stress, waterlogging and metal toxicity (Ceccarelli et al. 2010). Limited moisture stress (drought) can lead to a decrease in growth rate, increase in the duration of vegetative growth and change the photosynthetic rate and photosynthate distribution in maize. Short-term water deficits can cause substantial losses during rapid vegetative growth (28–32% of dry weight) and tasseling and ear filling stages (66–93%) (Cakir 2004). Long-term drought during pre-flowering stages had reduced the maize grain yield by 15–25% (Nesmith and Ritchie 1992). Heat stress alone can reduce the grain yield of maize up to 70% (Khodarahmpour and Choukan 2011). The excess moisture (waterlogging) stress for more than 3 days can reduce the maize grain yield by more than 40% (Li et al. 2011a, b, c). Similarly, salt stress, cold stress and metal ion stresses can reduce the maize yield substantially (Fahad et al. 2017).

3.2 Abiotic Stress Tolerance

Maize reaches its genetic yield potential in the optimal growing conditions. It requires 11–30°C temperature with 5–6 h of sunshine per day, moist soil rich in nutrients with a pH of 5.8–7.0, 200–450 mm of well distributed precipitation during its growth period (Yin et al. 2014). However, maize is exposed to various biotic and abiotic stresses that are responsible for substantial yield losses worldwide. Abiotic

stress refers to suboptimal climatic and/or edaphic factors that interfere with cellular homeostasis leading to impaired growth, reduced fitness and lower productivity. Abiotic stress can be transient (e.g., high temperature during mid-day) or chronic (high sodium stress in sodic soils). Abiotic stress at early vegetative growth affects cell division and expansion and slows down the growth which may not lead to much reduction in grain yield. But, abiotic stress at reproductive stage lead to substantial grain yield reduction (Mansouri-Far et al. 2010). Occurrence of multiple abiotic stresses, for instance, drought and heat causes more damage than their separate occurrences to the crops (Prasad et al. 2011). When abiotic stresses occur singly or in combination, though no visible symptoms are detected in early stages, significant alterations in physiology are already induced and affect the crop growth and development (Cramer et al. 2011), ultimately leading to reduction in grain yield of maize. Hence, maize productivity can be increased if grain yield loss caused by abiotic stresses is kept at minimum by adopting appropriate strategies.

The abiotic stress tolerance is the ability of plants to survive and produce harvestable yields when exposed to a stress or combination of stresses. Abiotic stress tolerance is a complex trait, and plants exhibit different adaptations at subcellular to organ levels. These include stomatal regulation, hormonal balance, activation of the antioxidant defense system, osmotic adjustment, and maintenance of tissue water status. As in case of other plants, maize too, cope with abiotic stresses by stress escape, tolerance or avoidance through the mechanisms of acclimation and adaptation acquired during natural selection. Maize plants have the ability to identify stresses and have developed response mechanisms to cope with. These mechanisms include stress escape (completing the life cycle before the onset of stress), stress avoidance and stress tolerance by alteration in morphological features and physiological processes. Several such physiological and biochemical mechanisms conferring improved tolerance have been reported for drought (Blum 1989; Foulkes et al. 2007), waterlogging (Rathore et al. 1996), low nitrogen stress (Cirilo et al. 2009), heat stress (Hasanuzzaman et al. 2013; Wani et al. 2016) and salt stress (Schubert et al. 2009; Jafar et al. 2012). The critical stages of various abiotic stresses, adaptation and/or tolerance mechanisms thereof have been summarized in Table 3.1.

3.3 Conventional Breeding Approaches for Abiotic Stress Tolerance

Limited moisture stress (drought), heat, salinity, waterlogging and cold are the major abiotic stresses faced by farmers. The agriculture activity is usually minimal in places where climatic extremes show regular occurrence while, in some places agricultural activity is intense and large population is dependent on agriculture but occurrences of climatic extremes are unpredictable leading to food insecurity. Till mid 1980s, globally, there were no concerted efforts towards development of abiotic stress tolerant genotypes in any crop plants including maize. In fact, it was not found necessary mainly because the most pressing issue at that time was to provide food to burgeoning population. However, efforts were made in the past to improve

Table 3.1 Consequences of abiotic stresses at critical stages of the plant growth

Abiotic stress	Critical stages	Consequences	Tolerance/adaptation	References
Drought stress	Flowering, grain filling	Reduced growth and vigour, Less pollens, low pollen viability, tassel blasting, delay in silking, increased anthesis silking interval, smaller seed, low yield	Reduced transpiration, improved water uptake, short anthesis silking interval, earliness, chlorophyll stability, deeper roots, reduced leaf area, osmotic adjustment	Ribaut et al. (2009), Hu and Xiong (2014)
Heat stress	Flowering, grain filling	Poor germination, stunted growth, decreased photosynthesis, increase in transpiration, cellular function affected, tassel blasting, pollen abortion, poor seed set, reduced seed weight	Membrane stability and reproductive viability, leaf cooling, change in membrane, composition, chaperones	Bita and Gerats 2013, Frey et al. (2016)
Waterlogging stress	Seedling, knee high, flowering, grain filling	Waterlogging for 48 h leads to impaired respiration, hypoxic/anoxic condition, energy crisis, cellular damage, root damage, wilting of shoot organs, lodging and mortality	Emergence of brace roots, root growth towards ground surface, more root porosity, low ethanol accumulation, rapid growth for avoidance, development of aerenchyma cells	Li et al. (2011a, b, c), Bailey-Serres et al. (2012)
Salt stress	Germination, plant stand establishment	Increased salt ions lead to cytotoxicity, reduced osmotic potential, poor germination, reduced shoot growth, reduced stomatal conductance, yellowing of leaves, burning of leaf tips	Reduced root ion uptake, limited root ion flux to shots, apoplastic acidification, vascular ion compartmentalization, osmotic adjustment, limited ion movement to transpiration stream	Munns and Tester (2008), Deinlein et al. (2014), Farooq et al. (2015)

(continued)

Table 3.1 (continued)

Abiotic stress	Critical stages	Consequences	Tolerance/adaptation	References
Cold stress	Late vegetative, early flowering	Membrane damage, low water potential, reduced germination, stunting, yellowing, leaf tip firing, delayed anthesis, reduced tassel size, silk size and pollen viability	Cold acclimation, dormancy, osmo-protection, altered membrane composition, increased compatible solutes, induction of stress protection genes, anthocyanin pigment accumulation	Morocco et al. (2005), Pietrini et al. (2002)

production and productivity under low moisture and low nitrogen stress. In major maize germplasm groups (Warburton et al. 2008). Most recently, single main focus was on improvement of tropical maize productivity of low-input rainfed agriculture practiced especially, in the countries with poor irrigation facilities. The main strategy followed was execution of stress tolerance breeding program at appropriate sites like managed drought and low nitrogen sites in main and dry seasons. It was followed by application of selection criteria like high grain yield, stress tolerance and other agronomic traits by imposing stress at different growth stages like flowering and grain-filling. The efforts have led to identification of key secondary traits through which selection for tolerance to abiotic stresses has been exercised. For example, ASI for drought and delayed leaf senescence for low nitrogen stress tolerance (Banziger et al. 2006). The results of such efforts have led to increased understanding of basic mechanisms which not only impart abiotic stress tolerance but also enhance the yield level under abiotic stress conditions. Theefforts have led to increase in yield by 25–30% under low nitrogen and also 1–3 tha^{-1} enhanced yield under managed low moisture stress conditions. Earlier breeding efforts coupled with strategic management options have offset yield losses by up to 40% (Thornton et al. 2009). This is possible since crop species including maize exhibit substantial genetic variability for tolerance to many climatic and edaphic stresses. Hence, breeding varieties that can withstand unpredictable climate extremes is one of the viable options in improving crop productivity under stress and to bring marginal land into cultivation. Maize being a cross pollinated crop, the phenomenon of heterosis has been exploited to a great extent. The deployment of hybrids helped in bringing down the effects and losses due to stresses to some extent because of their inherent ability to tolerate stresses by having better root system, early vigor, and quick growth compared to other types of varieties, though not specifically bred for stress tolerance. While breeding for abiotic stress tolerance, maize breeders can use two basic strategies, viz., indirect and direct approaches. In indirect breeding approach, the breeder exposes elite genotypes to an abiotic stress during evaluations at locations where stress conditions exist, even though they are not specifically bred for stress tolerance. In direct breeding approach, the selection is practiced under conditions where the abiotic stress occurrence is uniform and predictable. This can

be achieved by carrying out breeding activities under specific sites such as desert for drought and non-rainy period for drought and heat or by simulating a managed stress condition. Later the selection can be based on yield per se, implicated developmental traits for the target stress or plant water status (Acquaah 2012).

The procedure of breeding for abiotic stress tolerance in maize involves collection of diverse germplasm, identifying target stresses and environments, selection of elite cultivar as the recipient of stress tolerance, devising appropriate screens for each identified abiotic stress, screening of germplasm to identify donors and incorporation of tolerance mechanisms into the elite cultivar or into new cultivar through multi-environment evaluations (Bennet 2001). These steps require multi-center and multi-disciplinary maize improvement programs for phenotyping and cultivar development. Abiotic stress tolerant cultivars can potentially be developed by breeding methods such as introduction, selection, hybridization, and mutations. The abiotic stress tolerance is reported to be under complex inheritance and stress tolerant alleles are present at low frequencies in most elite breeding populations (Blum 1988). Hence, the development of stress tolerant lines requires accumulation of favorable alleles/genes either through mass selection and recurrent selection procedures or through ideotype breeding.

The earlier efforts of breeding for abiotic stress tolerance in maize indicated that genetic gain due to selection under stress condition may get reduce (Hallauer and Sears 1969), while some spill-over effect of selection under stress fee condition may appear under stress condition (Johnson and Geadelmann 1989). Therefore, most maize breeders practiced selection in high potential target environments followed by rigorous multi-location evaluation, which they considered to be the most effective approach for improving stress tolerance. However, theoretical concepts suggest that screening and testing should be done under representative production conditions and selection decisions should be made based on the relative economic importance of the crop both under stress- and stress-free environments (Rosielle and Hamblin 1981). Wherever the concept of managed stress was used while screening, genetic variation for abiotic stress tolerance have often been found (Edmeades et al. 1999; Banziger et al. 2000; Yadav et al. 2003). Thus, several strategies should be followed during selection for achieving success in breeding for abiotic stress tolerance: (i) selections and trials need to be conducted under optimal as well as managed stress conditions to incorporate abiotic stress tolerance at early breeding stage where genetic variance is large. Some earlier approaches exposed new varieties to stresses at later breeding stages where, variance was less with fewer genotypes (ii) abiotic stress should be critically managed to keep heritability of the target traits at higher level (Banziger et al. 2000) rather than using random stress conditions (iii) different stress levels are needed to analyze genotype x stress interactions in maize (Bolanos and Edmeades 1996; Banziger et al. 1997), since average stress intensities may not result in useful genotype x stress interactions (iv) use of secondary traits whose variance increases and heritability remains high under stress (Banziger and Lafitte 1997; Edmeades et al. 1998) (v) use of appropriate experimental design and statistical analysis to get higher heritability estimates (Gilmour et al. 1998) (vi) selection need to be practiced after adjusting the differences in flowering dates to reduce G × E (Banziger and Cooper 2001)

(vi) selection criterion should be good performance across the screening environments (Rosielle and Hamblin 1981), not for the minimum reduction in yield under stress compared to optimal conditions (Link et al. 1999) as this criterion is usually negatively correlated with yield.

The conventional approaches have been successful in enhancing the drought tolerance in commercial hybrids developed at both private and public sectors. The success of DTMA (Drought Tolerant Maize for Africa) and WEMA (Water Efficient Maize for Africa) projects highlights the potential of conventional breeding approaches in breeding for abiotic stress tolerance, though molecular breeding is an integral part of the project (Edmeades 2013). Under DTMA project, over 230 (63% hybrids and 27% improved OPVs) stress-resilient maize cultivars have been released in 13 countries in SSA during 2007–2015. In 2012, CIMMYT initiated HTMA (Heat Tolerant Maize for Asia) project in collaboration with national maize programs of Bangladesh, India, Nepal and Pakistan, and 15 private seed companies operating in Asia. This collaboration established 23 heat stress phenotyping sites in these countries. By 2017, this project has developed over 50 CIMMYT-derived elite heat stress tolerant maize hybrids which have been licensed to public and private sector partners for their deployment in the region (Cairns and Prasanna 2018).

3.3.1 Limitations of Conventional Breeding Approaches

The success of conventional methods of breeding for abiotic stress tolerance has so far been limited (Richards 1996). This is due to following factors: (i) abiotic stress tolerance is a complex trait involving complex pathways controlled by polygenes which make it too difficult to go for successful genetic manipulation while breeding (Wang et al. 2007) (ii) the primary criterion in breeding has been the yield rather than specific stress tolerance traits leading only to higher yield; (iii) phenomenon of G × E interactions further complicated the outcome (iv) infrequent use of simple physiological traits for measuring stress tolerance (iv) limitation in incorporating tolerance from unrelated highly tolerant species due to sexual barrier (v) most of the breeding efforts focused on one abiotic stress at a time while many a time combination of stresses (abiotic and biotic) are prevailing in a stressful environment (Mittler 2006) (v) the conventional breeding approaches require long time and more so in abiotic stress tolerance, mainly because, it is difficult to define target environment precisely, complex stress × environment interaction, and for want of precise screening methodology (Cooper et al. 1999).

3.4 Genetic Resources of Tolerance Genes

Genetic resources are the potential sources to improve any crop. These genetic resources are utilized for incorporation of useful genes underlying yield, biotic or abiotic stress tolerance into the target varieties/inbreds. The present section deals with the genetic resources available in maize, specifically, for tolerance to abiotic

stresses viz., drought, heat, cold and waterlogging conditions. The genetic resources may be classified as primary gene pool, secondary gene pool, tertiary gene pool or isolated genes/induced genes (Becker 1993). The primary gene pool comprises the crop species itself and other species that can easily be crossed with it. The secondary gene pool generally comprises of related species having less crossability with the target crop species. The tertiary gene pool usually has species that can only be used by employing special techniques like embryo rescue or other tissue culture techniques. The fourth class includes induced genes from mutation or isolated genes that may be derived from related or unrelated plant species, from animals or micro-organisms (Table 3.2, 3.3, 3.4 and 3.5).

Table 3.2 Primary gene pool

SN	Material	Abiotic stress	Genotypes	References
1	66 landraces from the gene banks at the International Institute of Tropical Agriculture (IITA), Nigeria and the Plant Genetics Resources Institute of Ghana	Drought and heat Stress	TZm-1159, TZm-1162, TZm-1163, TZm-1167, TZm-1472, TZm-1500 and TZm-1508	Nelimor et al. (2020)
2		Drought and combined drought and heat stress	TZm-1160, TZm-1162, TZm-1167, TZm-1440, TZm-1472, TZm-1486, TZm-1496 and TZm-1508	
3		Heat and combined drought and heat stress	TZm-1167, TZm-1162, TZm-1472, TZm-1508 and TZm-1506	
4		Drought, heat and combined drought and heat stress	TZm-1162, TZm-1167, TZm-1472 and TZm-1508	
5	36 early maturing landraces from Burkina Faso (6), Ghana (6) and Togo (21), and three drought-tolerant populations/ varieties from the Maize Improvement Program at the International Institute of Tropical Agriculture (IITA)	Drought stress	H-3505, TZm-1317, TZm-1307, GH-4859, GH-5756, TZm-1273, TZm-1353, TZm-1312 and TZm	Nelimor et al. (2019)
6		Heat stress	GH-4859, TZm-1353, TZm-1488, TZm-1441, TZm-1466, TZm-1473, TZm-1309, TZm-1325 and TZm-1317	
7		Drought and heat stress	GH-4859, TZm-1473, TZm-1325, TZm-1441, TZm-1466, TZm-1273, TZm-1551, TZm-1452 and TZm-1353	
8	Four genotypes	PEG induced drought stress	VIM147 and VIM396	Partheeban et al. (2017)
9	Nine genotypes	PEG induced drought stress	Arun-2, Rampur composite, & RL-105	Magar et al. (2019)
10	13 varieties of maize	Natural rainfed conditions	KDM 72, KG 2 and GM 6 had lowest ASI	Dar et al. (2018)

Table 3.3 Secondary gene pool

Trait	Crop wild relative	Putative cause of resistance/ tolerance	References
Waterlogging tolerance	*Z. nicaraguensis*	Ability to develop a barrier to radial oxygen loss in basal areas of adventitious roots under stagnant deoxygenated conditions	Abiko et al. (2012)
	Z. luxurians	Well-formed aerenchyma	Ray et al. (1999)
	Z. mays subsp. *huehuetenangensis*	Adventitious root system under flooding	Mano et al. (2005)
	Z. nicaraguensis	Root characters helping under water logging	Mano and Omori (2007)
	Z. nicaraguensis	Constitutive root aerenchyma formation	Mano and Omori (2013)
	Z. nicaraguensis	A higher capacity for adventitious root formation	Bird (2000), Iltis and Benz (2000)

Table 3.4 Tertiary gene pool

Trait	Crop wild relative	Putative cuase of resistance/tolerance	References
Drought tolerance	*T. dactyloides*	Deeply-penetrating root system	Gilker et al. (2002)
Salinity tolerance	*T. dactyloides*	Ability to conserve sodium in the leaves lowering water potential of leaves, maintaining the turgor pressure required for vegetative growth; and lowering the shoot/ root ratio	Pesqueira et al. (2003, 2006)
Waterlogging tolerance	*T. dactyloides*	Constitutive formation of root aerenchyma	Ray et al. (1998)
	T. dactyloides	Aerenchyma tissue that allows oxygen to penetrate to the distal regions of the roots	Glitz et al. (2013)

Table 3.5 Status of artificially induced/incorporated traits/genes

SN	Trait/gene	Tolerance to abiotic stress	Present status	References
1	*betA* from *E coli* glycine betaine	Drought tolerance	The transgenic maize plants accumulated higher levels of glycine betaine and exhibited higher tolerance to drought stress	Quan et al. (2004)
2	*ZmSDD1*	Drought tolerance	Overexpression of maize *SDD1* (*ZmSDD1*) improves drought resistance in *Zea mays* L. by reducing stomatal density	Liu et al. (2015a, b)
3	*GRMZM2G466563*	Abiotic stress	A member of calmodulin-binding super family, has been demonstrated to be an important signalling component in stress-induced cellular signal transduction pathway	Zielinski (1998)
4	*GRMZM2G172320*	Drought stress	involved in water stress signaling pathway	Yang et al. (2012)
5	Cytochrome P450 (*GRMZM2G092823*)	Drought stress	Encodes a key enzyme in ABA catabolism and plays a major regulatory role in controlling the level of ABA in plants	Kushiro et al. (2004)
6	*GRMZM2G09 0264*	Drought stress	GRMZM2G09 0264 is a Type-A Arabidopsis response regulator (ARR), which is rapidly induced by cytokinin and is a partially redundant negative regulator of cytokinin signaling	To et al. (2004)
7	*GRMZM2G163437*	Drought stress	GRMZM2G163437 is a subunit of ADP-glucose pyrophosphorylase, which is a key enzyme of the starch biosynthesis pathway	Sulmon et al. (2011)
8	*GRMZM2G179063*	Drought stress	GRMZM2G179063 is glucosyltransferase involved in glucuronoxylan	Keppler and Showalter (2010)

(continued)

Table 3.5 (continued)

SN	Trait/gene	Tolerance to abiotic stress	Present status	References
			biosynthesis and drought tolerance in Arabidopsis	
9	*GRMZM2G466563*	Drought stress	The putative calmodulin-binding protein (GRMZM2G466563) play important role in signal transduction and drought response	Alam et al. (2010)
10	*GRMZM2G428554*	Drought stress	Play important role in signal transduction and drought response	Perruc et al. (2004)
11	*GRMZM2G320269*, a peroxidase 27 precursor	Drought stress	Involved in the stress response	Kapoor and Sveenivasan (1988)
12	Plant invertases gene (*ivr2* encoding plant acid-soluble invertase)	Drought tolerance	Need to convert this into functional markers for incorporating drought tolerance in susceptible inbred lines of maize	Li et al. (2011a, b, c)
13	Arbuscular mycorrhizal fungi (AMF)	Cold stress	AMF might decrease the production and transmission of electrons under low temperature, and the cyclic electron flow process in chloroplasts was stimulated to protect plants against low temperature. The fungi also influenced transmission of electrons and production of phosphoric acid in mitochondria in response to low temperature. CO_2 assimilation capacity was affected and the tricarboxylic acid cycle was promoted by the adjustments in the glycolysis, pentose phosphate pathway, gamma-aminobutyric acid shunt pathway, and glyoxylic acid cycle to produce more adenosine	Li et al. (2020)

(continued)

Table 3.5 (continued)

SN	Trait/gene	Tolerance to abiotic stress	Present status	References
			triphosphate and raw materials for other metabolic pathways under low temperature	
14	QTL from teosinte	Waterlogging stress	Waterlogging tolerance has been incorporated into F_1 hybrid by QTL introgression	Mano and Omori (2007)
15	Introgression from *Tripsacum*	Drought tolerance	*Tripsacum*-introgression appears to confer larger, more robust root systems and overall increase in grain yield	Eubanks (2006)
16	Aerenchyma from *Z. luxurians*	Waterlogging tolernace	F_1 plants from the *Z. luxurians* × *Z. mays* cross had aerenchyma in well-aerated environments	Ray et al. (1999)

3.5 Diversity Analysis

3.5.1 Phenotype Based Diversity

In designing crossing programs and breeding systems, the information on the genetic diversity and relationships between maize genotypes is a pre-requisite. Over the past century, genetic diversity of the elite temperate maize pool has been gradually declining (Reif et al. 2005). However, tropical and subtropical inbred lines contain a greater number of alleles and gene diversity than temperate inbred lines, hence tropical and subtropical germplasm may be used to derive diverse inbred lines for temperate breeding programs.

The present day improved maize inbred lines on an average captured <80% of the alleles present in the landraces. This indicates that landraces can be a good source for widening the genetic base of maize (Vigouroux et al. 2005). Morphological and physiological adaptations have been seen in plants to cope with environmental stresses. However, evidence for natural or artificial selection of drought tolerance has rarely been examined in maize. *Tripsacum deltoids* or eastern gamagrass is known for its morphologic and metabolic characteristics-based drought tolerance (Gilker et al. 2002; Gitz et al. 2013). This species also showed increased water use efficiency and photosynthetic potential during water stress (Coyne and Bradford 1985).

Descriptions of the anatomical and other properties of wild relatives, especially of *Tripsacum* that contribute to its ability to withstand drought, come from studies of aerenchyma tissue in roots (Kemper et al. 1997), root penetration (Clark et al. 1996), and increased biomass (Risser et al. 1981). A number of studies indicated that compared to the Corn Belt Dent germplasm, maize originating from European Flint germplasm exhibited better cold tolerance (Strigens et al. 2013; Frascaroli and Landi, 2013; Revilla et al. 2016). Hence, creation of synthetic population was suggested based on the availability of inbred lines with favorable alleles of significant quantitative trait loci (QTL) for cold tolerance (Revilla et al. 2016).

In a study involving 15 maize inbred lines, those inbred lines with BSSS and Iowa Dent background showed more tolerance compared to the inbred lines with Lancaster background (Milivojevic et al. 2017).

3.5.2 Genotype-Based Diversity

The wide range of genetic variation existing in tropical maize germplasm, landraces and wild relatives has been indicated by the diversity studies at genetic, molecular, and functional levels. The molecular markers have been used for a variety of applications in maize diversity analysis like allelic frequency, (Reif et al. 2004), correlation between the parental diversity and hybrid performance, heterosis, and combining ability (Melchinger 1999, Betrán et al. 2003), molecular characterization of within or between populations (Warburton et al., 2002, Reif et al. 2004), determination of heterotic groups (Warburton et al. 2002, Xia et al. 2004, 2005), mapping of "phylogenetic" trees and diversity analysis (Lu and Bernardo, 2001; Warburton et al. 2002; Betrán et al. 2003; Liu et al. 2003; Reif et al. 2004; Xia et al. 2004, 2005), comparison of genetic diversity among different groups of maize germplasm (Liu et al. 2003; Tarter et al. 2004; Xia et al. 2005).

Based on the analysis of microsatellite markers in estimating the diversity and genetic structure in maize inbred lines from different temperate, tropical, and subtropical regions and CIMMYT, Mexico and IITA, Nigeria, five clusters could be formed, which aligned with the major breeding groups (Liu et al. 2003). It was defined as core subsets of inbred lines and can further be used in maize improvement and genetic research.

A study on 53 maize hybrids showed that diversity measures related to markers that were linked to yield QTLs explained 59–62% phenotypic variance (Vuylsteke 1999; Vuylsteke et al. 2000). CIMMYT based tropical, subtropical, and temperate maize breeding materials were characterized using microsatellite marker (Reif et al. 2004) in comparison with the landraces (Warburton et al. 2008). A paramount of genetic diversity was found within the breeding populations (Reif et al. 2004). Presence of a great reservoir of untapped genetic variation has been detected in all nucleotide polymorphism (SNP) markers provided a high- throughput tool for

evaluating and understanding large-scale maize germplasm and their geographical relationships.

A study was carried out on cold tolerance related traits, including the number of days from sowing to emergence, chlorophyll content and maximum quantum efficiency of photosystem II (F_v/F_m) in 406 recombinant inbred lines in a growth chamber under control conditions, and in the field at early and normal sowing. Association mapping based on genotyping with SNP markers revealed that 858 SNPs were significantly associated with all the above traits under cold conditions. Most QTLs were associated with chlorophyll and F_v/F_m (Yi et al. 2020). Among the species of *Zea, Z. nicaraguensis* with its habitat in Nicaragua is having higher waterlogging tolerance (Iltis and Benz, 2000). Mano and coworker identified the genetic components underlying water logging tolerance of *Z. nicaraguensis* viz., (1) the ability to grow adventitious roots; (2) formation of constitutive aerenchyma; and (3) tolerance to soil toxins (Mano et al. 2007, 2009; Mano and Omori 2008). It was concluded that *Z. nicaraguensis,* under stagnant deoxygenated conditions, has an ability to develop a barrier to radial oxygen loss (ROL) in basal areas of adventitious roots (Abiko et al. 2012). In addition, a progenitor species of teosinte, *Z. luxurians*, was also declared as a putative source of waterlogging tolerance (Omori and Mano 2007) followed by Eastern gamagrass (Ray et al. 1998). But, it may be noted that the efforts to introgress waterlogging tolerance into cultivated maize through traditional breeding approaches have not been successful due to the complex inheritance pattern of abiotic stress tolerance (Ray et al. 1999).

Using the population bulk fingerprinting strategy through simple sequence repeat (SSR) markers, ~800 global maize landraces/populations have been characterized under the Generation Challenge Program (GCP) project. The study led to the first-time assessment of genetic relationships among landraces/populations worldwide, compared to the country of origin, Mexico, besides indicating the possible migration routes of maize from Mexico to diverse continents and it was observed that number of alleles, gene diversity per locus, unique alleles per locus, and population structure all differ between germplasm groups (Warburton et al. 2008).

3.6 Utility of Molecular Tools

There is no doubt that the traditional breeding has brought significant improvement in yield gain and also developed several cultivars with tolerance to abiotic stresses. However, it is imperative that the traditional breeding cannot track genomic loci with small effects on the phenotype in population or progenies. Most often the small effect QTLs gets masked in environmental variance thus it is not possible to capture such variance to use in selection. Thus, it necessitates the use of molecular markers

to capture such small effect QTLs in regular selection process. The second important limitation of traditional breeding is that it cannot exercise selection for multiple stresses simultaneously unless selection pressure or stress conditions are created. Most often it is not possible to create multiple abiotic stresses simultaneously. On the contrary, traditional breeding would take more time for step-wise selection for multiple abiotic stress tolerance. Whereas with the availability of genomic sequence information in maize and for that matter in most of the crop plants, the implementation of advanced tools and techniques like MABB, MARS and GS would overcome all the above limitations of traditional breeding. Recently due to fast decreasing sequencing cost and availability of advanced data-analysis software tools, the field phenotyping is becoming relatively costlier. Thus, application of molecular tools and techniques can give preliminary information before field phenotyping of select advance material for final phenotyping in the field. Thus, integration of molecular breeding in regular breeding program would substantially reduce the cost involved in field phenotyping and at the same time it would increase the frequency of desirable recombinants providing more opportunity for selection by the breeders. Further, molecular breeding tools would also increase the genetic gain with simultaneous decrease in the breeding cycles required to develop new and improved genotypes with abiotic stress tolerance.

3.6.1 Utility of Molecular Mapping

The direct selection for abiotic stress tolerance is possible under in -situ by creating the stress conditions. But it requires perfect conditions for identification of either tolerance to abiotic stress or the expression of secondary traits like ASI and stay-green to name few associated traits with tolerance to abiotic stresses. But practically such perfect conditions are difficult to create because the effect of environment on different morphological traits especially quantitative traits is quite high which affects to a large extent the true expression of the traits. Thus, the selection for abiotic stress tolerance using secondary traits makes the selection process more complicated. Further, most of the abiotic stresses do not occur in isolation but in combination of two or more stresses. For example, effect of low moisture stress often coincides with high-temperature stress. In addition, the interaction among different abiotic stresses further masks the secondary trait expression. Thus, it further complicates the process of classical breeding for abiotic stresses tolerance. On the contrary, the heritability of abiotic stress tolerance and also the associated secondary traits is critical to obtain desirable genetic advance in the abiotic stress tolerance. It has been reported that creation of stress conditions and imposing selection under managed stress conditions may accelerate the breeding process by enhancing the genetic gain for abiotic stress tolerance (Banziger et al. 2006). However, such managed stress conditions require high initial

investment and infrastructures which may not be possible in most of the cases, especially in developing countries of the world. Further, the effect of abiotic stress on overall growth and development also varies from location to location and season to season. In that context, identification of appropriate representative locations to undertake abiotic stress resistance breeding for conducting multi-location evaluation trials is required. Further, identification of stable genomic regions which determine abiotic stress tolerance across seasons and locations is also most important to develop abiotic stress tolerant genotypes with wide adaptation. The use of molecular markers to map and identify gene(s) or genomic region(s) can facilitate to a large extent to develop stable abiotic stress tolerant genotypes.

The use molecular markers to track the specific genomic locations carrying gene (s) of interest in segregating generations and their association with economically important traits has led to the identification of numerous QTLs linked to the target traits. In maize, QTLs have been identified for most of the major abiotic stresses like moisture stress, temperature stress and nutrient stress. The major advantage of molecular markers is that they are not influenced by environment. Thus, they can be easily tracked over generations. Unlike conventional breeding, the establishment of stress environment is not required. Mapping can greatly facilitate the mobilization of stable and reliable QTLs or genomic regions from one genetic background to another genetic background through marker assisted backcross breeding (MABB) or marker assisted recurrent selection (MARS). Presently approaches like forward breeding using molecular markers and genomic selection (GS) are increasingly becoming cost effective new tools for breeders to accelerate the process of new cultivar development with increased tolerance to abiotic stresses. This will considerably reduce the time required to develop abiotic stress tolerant germplasm. Unlike conventional breeding where combining ability and heterosis are important considerations to develop commercially acceptable or viable products, marker assisted selection (MAS) can be directly used for value addition to the finished product further by introgression of abiotic stress tolerance.

3.7 Breeding Objectives and Selection Strategies for Abiotic Stress Tolerance

Climate resilience has become a buzz word in the scenario of changing climate. Historically, maize breeding has focused mainly on enhancing the yield levels. However, recently due to slow but unprecedented increase in the global average temperature, uneven distribution of rainfall over time across different agro-ecologies, declining resource base etc., have exerted pressure on breeder to enhance and sustain the production and productivity of crops including maize. It is the most urgent need to meet the growing food demand due to exponential increase in the population without compromising on the existing resource base. In fact, the demand has to be met by improving the quality of the existing resource

base to leave safe and sustainable ecosystem to the future generations as well. Thus, it is imperative to develop abiotic stress tolerant genotypes not only in maize but also in other crops to adapt to present challenges posed by different abiotic stresses threatening the global food security. The main breeding objectives are to dentify novel gene(s) and traits which can withstand abiotic stresses and also give higher yield by utilization of available genetic diversity. The breeding efforts also required to develop multiple stress resilient genotypes such as tolerant for drought + heat, drought + waterlogging, drought + nutrient stress, etc. Because, the impact of abiotic stresses and interaction among different abiotic stress differs depending on the geographical region and the type of cropping system prevailing there.

The perceived adverse impact on yield due to introgression of gene(s) determining tolerance to different abiotic stresses when such gene(s) are transferred from one genetic background to another could limit the utility of MAS, MARS, GS approaches for development of abiotic stress tolerant genotypes. Unlike breeding for higher yield, abiotic stress tolerance breeding should be targeted one for either one or combination of abiotic stress. Thus, targeted breeding could be the most appropriate strategy to develop new and high yielding abiotic stress tolerant cultivars. The type and degree of severity of stress determines the breeding goals, which often varies across locations and seasons. For example, the low moisture stress during *kharif* cannot be compared with that during *winter* season. Similarly, the low moisture stress in black soil vs red soils etc. Further the combined effect of drought and heat stress during *kharif* is different than that during *spring* or *summer* season. Therefore, before venturing into breeding for abiotic stress tolerance, it is important to decide the target area and season for desirable results. Abiotic stress tolerance being a complex trait the targeted pre-breeding for different abiotic stresses has been envisaged as the first step to progress towards development of abiotic stress tolerant genotypes (Gilliham et al. 2017). The degree of success and genetic gain largely depends on the phenotyping accuracies for different abiotic stresses. Thus, informed selection based on integration of high-throughput genotyping and phenotyping during abiotic stress breeding would substantially increase the frequency of desirable combinations.

3.8 Classical Mapping Efforts in Maize

Efforts have been made across discipline to understand the effects of different abiotic stresses on growth and development of maize which in turn affects ultimately the yield. It is imperative that phenotypic selection for resistance to different abiotic stresses alone may not yield tangible results in short-run. Because the adverse effects of different abiotic stresses on crop growth and development are highly influenced by environmental factors like soil type, rainfall, temperature, soil organic matter, soil biomass or micro-flora, relative humidity, etc. It is a known fact that abiotic stresses affect several metabolic pathways and the expression of various gene(s) and traits. In order to increase the efficacy of development of abiotic stress

tolerant genotypes, the objective selection of genotypes carrying gene(s) or secondary traits which are associated with abiotic stress tolerance is most important. In an effort to provide maize breeders the objective tools to facilitate the selection for abiotic stress tolerance several researches have tried to identify the key traits or gene(s) imparting abiotic stress tolerance. The classical example in maize is the anthesis silking interval (ASI) and its association with drought tolerance (Edmeades 1993). In general, under optimum growing conditions, the ASI would be around 2–3 days. However, under low moisture stress the ASI increases to >7 days and it can go up to 15 days. Sometimes under severe low moisture stress conditions, there will not be any silking leading to barrenness. Subsequently breeders used ASI as one of the important secondary traits for selection and development of low moisture stress tolerant genotypes. The meta-analysis of quantitative trait loci (QTLs) identified across different populations for ASI has shown that the number of QTLs specific to ASI are as high as 33 and with overlapping function with other traits like grain yield the number goes to 83 (Semagn et al. 2013). The QTLs for ASI have been mapped on almost all 10 chromosomes in maize. Similar to ASI, another important classical secondary trait across different crops with association to some of the abiotic stresses like drought and heat is the stay green. The QTL mapping of stay-green trait in maize has led to identification of several genomic regions conferring stay-green trait in maize (Wang et al. 2012; Zheng et al. 2009).

3.8.1 Association Mapping Studies

3.8.1.1 Studies on Linkage Disequilibrium

The term linkage disequilibrium (LD) was introduced to describe the degree of non-random association between pairs of loci. It can also be described as non-random co-segregation of alleles at two loci (Ersoz et al. 2009). Availability of either whole genome sequence information or large number of molecular markers including SNPs in public domain provides an opportunity for whole genome scan to map or tag the genomic regions determining economically important traits. The LD is the basic philosophy of all efforts related to gene tagging or mapping or construction of linkage maps. However, the utility of LD mapping has been extended from bi-parental mapping populations to group of unrelated individuals, most commonly called association panel. The association between alleles of genes originating from unrelated individuals indicates that they are in LD. Such associations provide a vital information on the functional relatedness of genes. In place of two alleles of a gene, if DNA sequence differences are associated with phenotype, it indicates the functional relationship between them. It is possible to map LD across the genome, the pattern of LD within the genome would actually depict the pattern of recombination. Since the association panel refers to group of unrelated individuals or population comprising unrelated individuals, the pattern of recombination actually depicts the historical recombination accumulated generation after

generations. The term 'unrelated' refers to individuals with distant lineage as getting unrelated individuals is practically very difficult in small breeding programs. The thumb-rule is that the group of individuals should possess genetic diversity.

LD between two alleles of a gene selected randomly can vary between pairs. Because, LD in addition to history of recombination it also largely influenced by mutation of an allele, genetic drift, selection and admixture. In general, the factors which affect the Hardy-Weinberg equilibrium also affect the LD. Therefore, before venturing into association studies one needs to understand genomic structure of the population or determine LD of the population in which association studies would be planned. LD differs from one species to the other and also from population to population within a species. The availability of ample number of molecular markers has led to an understanding on the LD in maize. Remington et al. (2001) conducted a study on pattern of LD in maize with respect to suitability of maze for association studies using 102 maize inbred lines (comprising temperate and tropical inbred lines representing global maize genetic diversity) and 47 simple sequence repeats (SSRs). This study has immense significance because the result of the study would indicate whether maize is suitable crop for conducting association studies to map complex traits with high-resolution. Based on the results it was observed that maize being highly cross-pollinated crop the LD decayed much faster. In the above study, 10% pairs of the 47 SSR markers have shown LD when all the 102 lines with diverse genetic background which indicates the suitability of SSRs to conduct association studies. Further, LD study using SNPs taken from gene sequence information has also shown the rapid decay in LD. The decay in LD differs from gene to gene and for some genes it is as less as 1500 bp which indicates that recombination does happen within gene also. Association studies in maize can be used for genetic dissection of complex traits with very resolution linkage distance. Several association studies have been conducted in maize to map several economically important traits that have shown secondary association with abiotic stress tolerance. LD can be used for association mapping by following two strategies namely, (1) the gene-based association analysis (target gene based LD studies) where the sequence of the gene(s) are available, and (ii) the whole genome association analysis (genome wide LD studies), where large number of markers are available. The brief account these two strategies are given below.

3.8.1.2 Target Gene-Based LD Studies

The QTL mapping studies using bi-parental studies have led to the identification of several genomic regions conferring tolerance to abiotic stresses. However, QTLs contain large genomic regions and identification of candidate genes requires follow-up studies like fine-mapping, map-based cloning and validation which are lengthy processes. Therefore, the availability of large number of SNP based molecular markers has accelerated the process of identification of candidate genes associated with polymorphic SNPs or haplotypes through association analysis or LD mapping. In maize, gene-based LD studies for abiotc stress tolerance are limited

as compared to biotic stress tolerance. Nonetheless in one of the gene-based LD study conducted on *nced* and *rab28* genes showed that the genes encode two important enzymes namely, 9-cis-epoxycarotenoid dioxygenase (NCED) and abscisic acid (ABA)-responsive gene protein 28 (RAB28) which are known to be involved in ABA biosynthesis and ABA induced drought tolerance, respectively. ABA is known to play key role in multiple stresses by modulating the expression of several biological processes from germination to seed maturation. Thus, it is very critical, if LD studies focus on such key compounds to understand the mechanism involved in tolerance to abiotic stresses. In the above study several polymorphic sites have been identified in *nced* and *rab28*gene sequences by comparing the gene sequences across diverse set of maize genotypes. Key morphological and yield and yield related traits along with stress responsive traits under moisture stress were studied. The analysis of results has identified association of several key polymorphic sites in the gene sequences with as high as ten key traits with significant variation under moisture stress conditions (Su et al. 2011). Similarly, gene-based LD analysis for drought tolerance has identified several candidate genes which encodes for ABA receptors and impart low moisture stress tolerance. The study has identified key genes namely, *ZmPYL8*, *ZmPYL9* and *ZmPYL12* for drought tolerance in maize (He et al., 2018). Thus, the gene-based LD studies on such key genes would further facilitate identification and validation of abiotic stress tolerance genes across different genetic background and subsequent mobilization via linked molecular markers into otherwise elite cultivars to infuse abiotic stress tolerance. The advancement in sequencing technologies has brought down the cost involved in genotyping. The possibility of generating genotype specific sequence information through genotyping by sequencing (GBS) due to continuous decrease in the cost of sequencing has further accelerated the association studies and largely shifted from gene-based LD studies to genome-wide association study (GWAS).

3.8.1.3 Genome-Wide LD/Association Studies (GWAS)

A GWAS in maize has led to identification of genes conferring resistance to different abiotic stresses. For instance Wang et al. (2016) has identified *ZmVPP1* gene conferring resistance to low moisture stress (drought) in maize. The gene was found to encode for vacuolar H+ pyrophosphatase. The sequence analysis of *ZmVPP1* indicated that 366-bp insertion in the promoter region confers drought-inducible expression of the gene in drought tolerant lines. It was also reported that the insertion regions contain three MYB transcription factor (TF) binding *cis*-elements. In other studies, conducted by Morosini et al. (2017) three candidate genes conferring for nitrogen use efficiency were identified. It was found that candidate genes were associated with transcription regulation, synthesis of monophosphate guanidine and biosynthesis of sphingolipids. Low moisture stress tolerance found to be very complex in nature. Several associated traits namely grain yield, flowering time ABA, root traits etc. which were found to be associated with drought tolerance in maize have been studied extensively through QTL mapping (Wang and Qin 2017).

Efforts have also been made to map genomic regions which determine drought tolerance. Yuan et al. (2019) conducted GWAS using 300 tropical inbred lines and 381,165 GBS SNPs for grain yield and flowering time under well-watered, drought stress, heat stress and combined drought and heat stress conditions. The study has identified 46 candidate genes with differential expression under well-watered and drought stress conditions. Another important abiotic stress is cold stress and maize is highly sensitive to low temperature. Whenever temperature goes below 10 °C, the germination and vigor gets affected and hence, cold tolerance during germination is very crucial. Hu et al. (2017) conducted GWAS for chilling tolerance using 241 inbred lines and >2.2 million SNPs. The study has identified as high as 17 SNPs associated with cold tolerance, out of which 7 SNPs were located on the candidate genes itself. Out of 17 SNPs, 5 SNPs were directly hit the QTLs which were previously reported for cold tolerance in maize. On the contrary high temperature tolerance during flowering is also crucial for proper seed-set. Gao et al. (2019) conducted two studies namely QTL mapping using 237 recombinant inbred lines (RILs) and GWAS using 261 diverse maize inbred lines. The number of SNP markers used in QTL mapping and GWAS is 8329 and 259,973, respectively. The study has identified four QTLs and 42 SNPs associated with 17 genes determining cold tolerance. Based on the information on candidate genes, the calcium signaling pathway was found to play a central role in cold tolerance. Thus, GWAS strategy to identify genomic regions determining the tolerance to different kinds of abiotic stresses would further accelerates the rapid identification of key genes and their deployment in otherwise elite genetic backgrounds though markers assisted selection (MAS).

3.8.2 Application of Association Studies for Germplasm Enhancement

The germplasm enhancement covers two vital but basic aspects which includes increasing the frequency of desirable alleles as well as reducing the frequency of deleterious alleles in the germplasm affecting various traits. Germplasm enhancement is a continuous process. It is evident from the above section that association studies do not have limitations with respect to inclusion of germplasm in the association studies. Association study help to identify diverse alleles or multiple alleles of the same gene distributed in the germplasm in varying frequencies. It also accounts for the historical recombination and helps in identifying all those genomic regions which have maintained the integrity of genomic regions with tight linkage with phenotype. The desirable alleles may be further mobilized into different genetic background. Bringing desirable alleles distributed in the germplasm into the active germplasm would not only enhance the overall average performance of germplasm as a whole but also increase the frequency of desirable alleles. Thus, it can contribute substantially towards the goal of germplasm enhancement.

Association studies have become an important tool in recent years which is being extensively being used for mapping complex traits with high resolution.

Association mapping holds distinct position in the pools of various tools and techniques available with breeders to identify useful and genetically divergent alleles from large pool of germplasm. The large-scale implementation of marker assisted introgression of gene(s), which are identified through association studies in routine breeding programs, would not only increase the genetic gain but also enhance the value of available active germplasm. The utility of association studies would be more relevant while dealing with complex traits like tolerance to abiotic stresses as it helps to identify candidate genes or functional alleles for different traits in most of the cases. The availability of SNP markers in large number and their association with large number of economically important traits would provide a platform for implementation of genomic selection and markers assisted recurrent selection as part of germplasm enhancement strategy through recurrent selection. Abundant SNP marker resources are available in maize and extensive work has been also done to generate and catalogue most informative SNP markers for whole genome scan which greatly facilitate to capture the allelic variants of most of the economically important traits.

3.9 Molecular Mapping of Stress Tolerant Genes and QTLs

3.9.1 Evolution of Various Marker Types: RFLPs to SNPs

The development of new and improved cultivars of maize broadly comprised of two important steps viz., (i) development of inbred lines, (ii) development of hybrids. Use of marker systems and tools as an integral part in selection process during cultivar development has evolved over a period of century. The process of selection always based on some references which could be a trait or group of traits. However, the phenotype is the expression of genotype in the particular environmental context. But phenotype is influenced by the changes in the environments. More often aberrations in the environment mask the true expression of phenotype which affects the selection process. Thus, the search for stable reference points was started and it started with morphological markers like association of simply inherited traits which are not generally influenced by changes in the environment (presence of pigmentation, shape of the organs, presence of spines or ligules etc.). However, such morphological features are limited in number. Furthermore, often, there are no perfect marker systems. Even though some simply inherited traits show stable expression, they express at particular stage and one has to wait till that particular stage to come. Therefore, the search for new kinds of marker systems has started mostly with the objective to find stable, stage neutral, reliable and easy to score marker systems. It all started with biochemical markers like isozyme markers, the actual expression of phenotype, they are more reliable and robust. But the major limitation is that they are less in number.

The discovery of genetic material *i.e.* deoxyribonucleic acid (DNA), ability to unravel the sequence information of genetic material, tagging the genetic material with radioactive elements, artificial synthesis of genetic material and the discovery of restriction enzymes together have paved the way to search for new kinds of markers like restriction fragment length polymorphism (RFLP). The second remarkable advancements like amplification of genetic material through polymerase chain reaction (PCR) and further advancement in the sequencing techniques has led to the development of different types of PCR based molecular markers like, randomly amplified polymorphic DNA (RAPD). Subsequently, another type of marker, amplified fragment length polymorphism (AFLP) was developed. AFLP was developed by using the principles of RFLP and RAPD. The advancement in sequencing technologies, accumulation of genomic (DNA) sequences, short sub-sequence of a cDNA sequence called expressed sequence tag (EST) in public domain and development of and maintenance of sequence data base by The National Center for Biotechnology Information (NCBI) has led to development of robust, reliable, easy to score, PCR-based markers like, sequence tagged microsatellites (STMS) or simple sequence repeats (SSRs) and their modifications like inter simple sequence repeats (ISSRs), sequence tagged sites (STSs). As the new marker systems became easily available in different crops the advancement in the earlier marker systems has also made them more reproducible, robust and reliable. Subsequently, important RAPD markers were converted into sequence-characterized amplified region (SCAR) markers or cleaved amplified polymorphic sequences (CAPS). The continuous advancement in sequencing technologies and reduction of cost of sequencing and also completion of whole genome sequencing projects in different crops, including maize has opened the opportunity to use millions of single nucleotide markers (SNPs) for deciphering the differences in sequences between genotypes and their correlation with differences in phenotypes. However, SNP markers should not be confused with earlier markers like RFLP, which also unravel the sequence differences at restriction site but here sequence information-based SNPs are in very large number. The reason for more acceptability of SNPs over other types of molecular markers is only because of their availability in large number which provides an opportunity to dissect the genomic regions at very high resolution to study the association between gene(s) and traits. Most often because of large number, SNPs do provide functional relationship with phenotype and most of the time they land in the candidate gene itself. The further mobilization of such important key gene(s) across different genetic background is very easy.

3.9.2 Mapping Populations

The application of molecular markers to determine the genomic regions and to establish the association between genotype and phenotype requires different types of genetic resources like mapping populations. Development of mapping populations are equally important as that of molecular markers; without mapping populations, it

is virtually impossible to study the association between genotype and phenotype. The basic principle of mapping is the identification of contrasting phenotypes and genotypes. Further, identification of polymorphic molecular markers between the contrasting genotypes possessing contrasting phenotypes is very crucial for further association studies. The most common mapping populations used for construction of linkage map and also QTL mapping are bi-parental mapping populations. The example of mapping populations is F_2, backcross derived mapping populations, recombinant inbred lines (RILs) and near isogenic lines (NILs). All the above listed mapping populations can be derived from F_1 hybrid ($P_1 \times P_2$), where P_1 and P_2 represents female and male parents involved in the development of F_1 cross respectively. The F_2 mapping population can be developed by simply selfing the F_1 hybrid. The backcross derived mapping populations can be derived by backcrossing F_1 with either of the parents. If F_1 is backcrossed with female parent (P_1), it can be designated as BC_1P_1, whereas F_1 backcrossed with male parent (P_2) can be designated as BC_1P_2. If F_1 is advanced for 5–6 generations to achieve >98% homozygosity at genetic level by repeated selfing, then the progenies of such advancement are termed as recombinant inbred lines (RILs). The near isogenic lines can also be used for mapping or tagging the limited number of gene(s) and also can be used to study the effect of genetic background on the expression of gene(s) or QTLs.

It has been observed that each of above mapping populations have their own advantages and disadvantages. For example, the map resolution using F_2 mapping populations is very coarse because of a smaller number of recombination cycles. The QTLs identified using F_2 mapping population mostly not directly be used in MAS. But the QTLs identified using RILs can be used in MAS. However, many times it is not possible to identify the candidate genes through QTL mapping using RILs. In addition, development of bi-parental RIL mapping populations is time-consuming and resource demanding as well. The F_2 mapping populations cannot be maintained due to heterozygous condition.

In order to overcome the above limitations or the other kind of mapping population like association mapping (AM) population or panel was proposed. The concept of association mapping population has actually given opportunity to undertake mapping with limited resources as well. Association mapping populations overcome almost all the limitations of bi-parental mapping populations. However, the major draw-back is the existence of unknown population structure and false-positive associations.

Subsequently the concept of community mapping resources in the form of nested association mapping (NAM) populations has further given the opportunity take the advantages of both bi-parental mapping populations as well as association mapping by simultaneously overcoming the limitations associated with each kind of mapping populations. Later multi-parent advance generation inter-crosses (MAGIC) was proposed to overcome some of the limitations associated with NAM. The major advantages of MAGIC over all other mapping populations are its known population structure and more number of recombination events. Thus, it provides an opportunity to sample all alleles multiple times, which increase the statistical power to estimate the effects of alleles.

3.9.3 Mapping Software

Two important aspects namely, availability of molecular markers and mapping populations have been already discussed. However, the third important aspect is the statistical tools and techniques to make analysis of the genotypic and phenotypic data to arrive at the right conclusions. The simultaneous advances in statistical tools and techniques along with new software packages to handle huge data points, which is humanly impossible, has made all kinds of mapping experiments possible. It is practically not possible to enumerate all software packages which are being used in linkage map construction, QTL mapping and also association mapping experiments. The researchers can have multiple options; both paid and free software packages. These packages are available in the public domain. It is purely the choice of researchers to choose any of the available statistical packages for analysis. It all depends on the type of results one is interested and also the type of data available with the researchers to perform the analysis. Some of the most popular statistical packages used for construction of linkage maps are Mapmaker, JoinMap, AntMap, MergeMap, MMAPPR, MadMapper and Tassel (Boopathi 2013). However, it is not limited to above mentioned software, but numerous other softwares are available in public domain.

Several algorithms have been developed for analysing the data and they differ depending on the input data. Multiple statistical techniques have been developed to suit various types of data, so researchers can have multiple options for proper analysis and interpretation.

3.10 Marker Assisted Breeding for Tolerance Traits

3.10.1 Gene Pyramiding

Gene pyramiding aims at stacking/accumulating several resistance genes/QTLs and is a powerful technique for transferring several desired genes or QTLs from different inbred parents into a single genotype. Pyramiding of desired gene is possible in the shortest possible time (two to three generations) with recent advances in molecular markers techniques as compared with conventional breeding approaches that takes a minimum of six generations to recuperate 99.2% of the recurrent parent genome (Perumalsamy et al. 2010; Suresh and Malathi 2013; Hasan et al. 2015). However, two components are most important for the utilization of MAS to breed for drought tolerance: (1) Identification of informative QTLs for the component traits, (2) Introgression of the identified QTLs through suitable MAS approaches. Drought tolerance is a complex trait made up of combination of several quantitative traits. These traits may show high level of epistatic and environmental interactions. Assessment by quantifying the phenotypic variation explained by the QTLs and their interactions are important issues, as the effects are confounded with

study design, genotyping, marker coverage, selection of component traits, efficient phenotyping, QTL mapping models, methodologies and so on. Any deviation on the above-mentioned factors would significantly alter the number of QTLs detected and their phenotypic contribution. These variations may distort the understanding of marker-trait association. In addition to this, though informative QTLs are identified, practically, pyramidding many QTLs through MABC remains a herculean task. So, it is the practical difficulty and transferring one or two major QTLs through introgression breeding, do not provide the expected level of trait expression. A QTL identified with epistatic interaction lose the effect in the absence of its counterparts during the expression. Hence these factors are considered during the QTL mapping experiments and their possible introgression (Nepolean et al. 2018) (Fig. 3.1; Table 3.6).

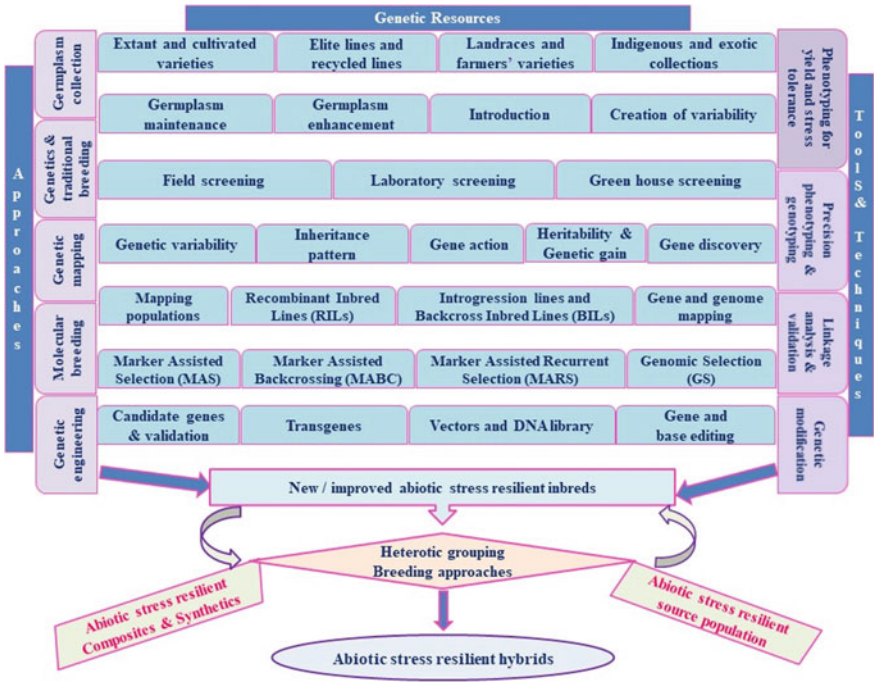

Fig. 3.1 Integration of traditional and innovative breeding approaches for developing stress resilient maize hybrids

Table 3.6 Molecular tools for in silico analysis of genes/QTL

S. No.	Name and link	Description
1	MetaQTL http://bioinformatics.org/mqtl/	A java package designed to perform the integration of data from the field for gene mapping experiment
2	BioMercator http://cms.moulon.inra.fr/index.php?option=comcontent&task=view&id=13&Itemid=43	Genetic maps and QTL integration
3	CMTV http://www.ncgr.org/cmtv/	An integrated bioinformatics tool to construct consensus maps and compare QTL and functional genomics data across genomes and experiments
4	QTL-Finder http://gqtl.maizecenter.cn	A bioinformatics tool for QTL integration, comparison and candidate gene discovery across genomes and experiments
5	TWINSCAN http://mblab.wustl.edu/query.html	Improved gene prediction performance for maize and rice
6	GDPC http://www.maizegenetics.net/gdpc/index.html	The genomic diversity and phenotype connection (GDPC) simplifies access to genomic diversity and phenotype data, thereby encouraging reuse of this data. GDPC accomplishes this by retrieving data from one or more data sources and by allowing researchers to analyze integrated data in a standard format. GDPC provides access to genomic diversity data such as SNPs, SSRs, and sequences, and phenotypic data that may be collected in field, genetic, or physiological experiments
7	TASSEL http://www.maizegenetics.net/index.php?page=bioinformatics/tassel/index.html	A software package to evaluate trait associations, evolutionary patterns, and linkage disequilibrium
8	PowerMarker http://statgen.ncsu.edu/powermarker/	A comprehensive set of statistical methods for genetic marker data analysis, designed especially for SSR/SNP data
9	RepMiner http://jestill.myweb.uga.edu/RepMiner.htm	Takes a graph theory approach to the identification and assembly of transposable elements from small DNA fragments resulting from subcloning bacterial artificial chromosome libraries
10	SPAGeDi http://www.ulb.ac.be/sciences/ecoevol/spagedi.html	Spatial Pattern Analysis of Genetic Diversity (SPAGeDi) is a new computer package—replacing AutocorG that was distributed to a limited extent—primarily designed to characterize the spatial genetic structure of mapped individuals and/or mapped populations using genotype data of any ploidy level

(continued)

Table 3.6 (continued)

S. No.	Name and link	Description
11	Structure http://pritch.bsd.uchicago.edu/structure.html	A free software package for using multilocus genotype data to investigate population structure
12	TEnest http://bak.public.iastate.edu/tenest.html	Automated chronological annotation and visualization of maize nested transposable elements
13	Bayesian QTL/Multimapper	Bayesian multi-locus models (i.e., Bayesian whole-genome regression or Bayesian alphabets) in identifying genetic determinants (including epistasis) and prediction of individual's genetic value (merit/risk) to the quantitative, qualitative and function-valued human, plant and animal traits based on genome-wide sets of molecular markers

3.11 Genetic Engineering for Resistance to Abiotic Stresses

3.11.1 Drought Resistance

3.11.1.1 Cytokinin Oxidase

Ckx1 gene encodes cytokinin oxidase in maize, which regulates cytokinin levels. Transgenic expression of a transcriptional fusion of the *Ckx1* promoter to the *E. coli glucuronidase* reporter gene in maize revealed that the gene is expressed in the vascular bundles of kernels, seedling roots, and coleoptiles. It has been shown that *Ckx1* gene expression is inducible in various organs by synthetic and natural cytokinin in normal conditions but induced by abscisic acid during stress conditions (Brugière et al. 2003).

3.11.1.2 Phytoene Synthetase

Phytoene synthetase enzyme catalyzes first step of carotenoid biosynthesis and involved in ABA biosynthesis. Phytoene synthetase gene in maize and other members of family Poaceae have three paralogs, *PSY1*, *PSY2* and *PSY3* and all encodes functional enzymes (Li et al. 2008a, b). *PSY3* is associated with root carotenogenesis and needed for drought and salt stress-induced production of ABA (Li et al. 2008a, b), and *PSY1*, encoded by the Yellow1 (Y1) locus, is required for endosperm carotenoid accumulation.

3.11.1.3 Phosphatidylinositol (PtdIns) Synthase

PtdIns synthase is a key enzyme in the phospholipid pathway which catalyzes the formation of PtdIns. Despite being a structural component of cell membranes, PtdIns are also the precursor of the phospholipid signal molecules which regulate plant response to various environment stresses. Transgenic maize constitutively over-expressing or under-expressing PIS from maize (*ZmPIS*) downstream to maize ubiquitin promoter were developed and exposed to drought stress. The drought stress tolerance of the *ZmPIS*sense transgenic plants was enhanced significantly at the pre-flowering stages compared with wild type maize plants. These findings infer that *ZmPIS* regulates the plant response to drought stress by altering membrane lipid composition (Lu et al. 2013).

3.11.1.4 Phosphatidylinositol-Specific Lipase C

The *ZmPLCl* gene cloned from maize (*Zea mays* L.) encoded a PI-PLC and its expression in maize roots found to be up-regulated in dehydration conditions. The individuals expressing *ZmPLCl* transgenes in sense orientation showed better osmotic adjustment, higher relative water content, increased photosynthesis rates, less lipid membrane peroxidation, lower percentage of ion leakage and higher grain yield than that of wild type; whereas those expressing the antisense transgene showed inferior quality. This showed that the enhanced expression of sense *ZmPLCl* improves the drought tolerance in maize (Wang et al. 2008).

3.11.1.5 ARGOS Protein

The phytohormone ethylene controls plant growth and development as well as plant response to abiotic stress. Recent findings have shown that altering ethylene biosynthesis and signaling can enhance plant drought tolerance. Novel negative regulators of ethylene signal transduction in Arabidopsis (*Arabidopsis thaliana*) and maize (*Zea mays* L.) are encoded by the *ARGOS* (auxin-regulated gene involved in organ size) gene family (Shi et al. 2015). Genetic analysis suggested that the *ZmARGOS1* transgene acts between an ethylene receptor and *CONSTITUTIVE TRIPLE RESPONSE1*, affecting ethylene perception or the early stages of ethylene signaling (Shi et al. 2015).

3.11.1.6 ACC Synthases

ACC synthases (ACSs), catalyze the rate-limiting step in ethylene biosynthesis. In transgenic plants, ethylene emission levels were reduced approximately by 50% when compared with non-transgenic controls. It indicated that transgenic plants had significantly increased grain yield over the controls, with the best event having a

0.58 Mg/ha (9.3 bushel/acre) increase after drought exposure during flowering period. Also, flowering traits showed consistent decrease in the anthesis-silking interval and a concomitant increase in kernel number/ear in transgene-positive plants versus controls. It demonstrated that the grain yield of maize under abiotic stress conditions can be improved by down-regulating the ethylene biosynthetic pathway (Habben et al. 2014).

3.11.1.7 LEA Protein

LEA gene(s) can be used as potential candidates to enhance stress tolerance in plants. Constitutive expression of *Rab28 LEA* gene in maize plants resulted in sustained growth upon polyethylene glycol (PEG)-mediated dehydration. Under osmotic stress, it was observed that transgenic seedlings recorded increased leaf area and root biomass, less chlorophyll loss and production of lower Malondialdehyde (MDA), enhanced relative water content (RWC) in comparison to wild-type plants. In addition, transgenic seeds exhibited higher germination rates than wild-type seeds under water deficit conditions (Amara et al. 2013). In another study, a putative group 5C LEA gene *ZmLEA14tv* from *Z. mays* cv. Tevang1, was cloned and expressed in tobacco and maize. During drought exposure, the *ZmLEA14tv*-expressing maize plants showed enhanced the seed germination and survival rate as compared to non-transgenic control plants (Ganther et al. 2020).

3.11.1.8 Protein Kinases

Constitutive expression of tobacco *MAPKKK* (*NPK1*) in maize plants have shown improved drought tolerance with significantly higher photosynthesis rates. Moreover, the kernel weight in drought-stressed transgenic plants was similar to those under well-watered conditions, while in drought-stressed non-transgenic control plants, the kernel weight was significantly reduced when compared with their non-stressed counterparts (Shou et al. 2004).

3.11.1.9 Choline Dehydrogenase, Glycine Sarcosine Methyltransferase (ApGSMT2) and Dimethyl Glycine Methyltransferase (ApDMT2)

Glycine-betaine plays an important role in the protection mechanism of many plants under various stress conditions. Transgenic maize plants accumulated higher levels of glycine-betaine when it was transformed with the *beta* gene encoding choline dehydrogenase and were more tolerant to drought stress than wild-type plants at germination and the young seedling stage. Above all, the grain yield of transgenic plants was significantly higher than that of wild-type plants after drought treatment (Quan et al. 2004). Maize plants with an increased ability to synthesize

glycine-betaine (GB) were produced by introducing two genes, dimethylglycine methyl transferase (*ApDMT2*) and glycine sarcosine methyl transferase (*ApGSMT2*) from *Aphanothece halophytica*. The transgenic plants showed an increased accumulation of sugars and free amino acids, greater chlorophyll content, lower Malondialdehyde and electrolyte leakage, a higher photosynthesis rate and biomass compared to the wild-type plants. To enhance abiotic stress tolerance in maize and other crops, the co-expression of *ApGSMT2* and *ApDMT2* can be used as an effective approach (He et al. 2013).

3.11.1.10 Vacuolar H+-Pyrophosphatase

The heterologous expression of *TsVP*gene from a dicotyledonous halophyte *Thellungiella halophila* improved the drought resistance in maize. The transgenic plants had higher V-H+-PPase activity, enhanced seed germination percentage, well developed root systems, less cell membrane damage followed by higher solute accumulation, compared to wild type plants under osmotic stress. Upon drought exposure, transgenic plants showed shorter anthesis-silking interval, less growth retardation and produced high grain yields (Li et al. 2008a, b).

3.11.1.11 Pyramiding of Choline Dehydrogenase and Vacuolar H+-Pyrophosphatase

Enhancement of resistance to abiotic stress, transgenic maize plants expressing both *betA* (encoding choline dehydrogenase from *E. coli*) and *TsVP* (encoding V-H+-PPase from *Thellungiella halophila*) were developed by hybridization. The pyramided transgenic plants had higher H+-PPase activity and glycine-betaine contents compared with the parental lines, which had either *betA* or *TsVP*, and contained greater solute accumulation, higher relative water content (RWC) and lower cell damage under drought stress condition. It has been suggested that co-expression of the two genes involved in different metabolism pathways in pyramided transgenic maize can help to improve the drought resistance over their parental lines that contained either of single transgene (Wei et al. 2011).

3.11.1.12 Trehalose-6-Phosphate Phosphatase

Trehalose-6-phosphate phosphatase (TPP) produces trehalose from T6P. T6P acts as a signal of sucrose status and a powerful growth regulator mediates sucrose

utilization with growth and development in relation to environmental conditions. In previous studies it has been accomplished that by increasing expression of *TPS* and/or *TPP* to improve drought tolerance in model plants, rice and potato. A rice *TPP* was over-expressed in developing maize ears downstream to floral promoter. Over-expression of *TPP* increased both kernels set and harvest index. Field data at several sites and over multiple seasons showed that in transgenic plants yields got enhanced from 9 to 49% under non-drought or mild drought conditions, and from 31 to 123% under more severe drought conditions, when compared with non-transgenic controls (Nuccio et al. 2015).

3.11.2 Combined Drought and Salt Stress Resistance

3.11.2.1 CBL (Calcineurin B-like Protein)

A total of 12 *CBL* genes from maize were identified from the maize genome. One of the *CBL* gene, *ZmCBL9*, which can be induced by salt, dehydration, glucose and abscisic acid (ABA) treatments was over expressed in Arabidopsis. The expression of genes in the ABA signaling, biosynthesis and catabolism pathways were negatively regulated by *ZmCBL9* gene. The *ZmCBL9* protein interact with 8 maize CIPKs. The *ZmCIPK* genes were up-regulated by different stress treatments, including dehydration, glucose, salt, low potassium and ABA. These results suggest that *ZmCBL9* may interact with various *ZmCIPKs* to regulate the abiotic stress and ABA response signaling in plants and can be used as a potential candidate for development of abiotic stress tolerant crops (Zhang et al. 2016).

3.11.2.2 *HVA1* (*Hordeum vulgare* Abundant Protein)

Transgenic maize developed using *HVA1* gene and/or bacterial mannitol-1-phosphate dehydrogenase (*mtlD*) gene, downstream to *Act15* promoter. Transgenic plants with a combination of the *HVA1+mtlD* showed greater plant survival and higher leaf relative water content (RWC) compared with their single transgene expressing plants and with their control plants. plants expressing the *HVA1+mtlD* showed higher fresh and dry shoot and dry root biomass as compared with plant expresssing single transgene and with their control plants, when exposed to various salt concentrations and also higher levels of mannitol was accumulated in the leaves of plants expressing the *mtlD*. These findings support the effectiveness of co-expression of two heterologous abiotic stress tolerance genes in maize and other agronomically important crops (Nguyen and Sticklen 2013).

3.11.3 Combined Drought and Heat Tolerance

3.11.3.1 MYB Transcription Factors

To improve the tolerance to heat stress and drought, the *OsMYB55* gene from rice was over-expressed in maize. Drought tolerance was evidenced by the reduced leaf damage and higher plant biomass in transgenic plants in comparison to wild type plants when subjected to individual drought or heat stress or during imposition of combined stresses. Transcriptomic analysis of transgenic *OsMYB55* maize recorded the upregulation of that several genes induced by heat stress in wild type plants. Up-regulation of genes upon heat and drought exposure, suggested probable gene association of expression with the stress tolerance in the transgenic maize. Hence, it was concluded that expression of *OsMYB55* can improve combined heat and drought tolerance in maize (Casaretto et al. 2016).

3.12 Bioinformatics as a Tool for Studying Abiotic Stress Tolerance in Maize

The sequencing of maize genome (Schnable et al. 2009) has opened up the possibility of carrying out whole genome studies for understanding adaptation response of maize against various abiotic stresses. The enormous genomics, transcriptomics and proteomics data are being regularly catalogued in various databases. An illustrative list of relevant databases for maize abiotic stress research is provided in Table 3.1. Bioinformatics is an important tool in genomic analysis of maize germplasm with unique stress tolerance trait. The various expression databases and tools provide opportunity of comparative and massively parallel analysis of various stress responsive genes, alleles, transcripts, small RNAs and proteins in maize germplasm. Often the bioinformatic study is result of previous lab and field work related to stress experiments or the *in silico* study itself paves way for further wet lab validation and field work (Table 3.7).

3.13 Social, Political and Regulatory Issues

The techniques like gene editing, new biotechnological tools in agriculture drew more attention by the policymakers, environmentalists and even consumers questioning the safety, preference, biodiversity alteration and disturbance in the natural balance. Therefore, any technique and product, for that matter, should be received and placed well in social, economic and political aspects. Experiences across the world indicate the progress of gene editing is largely determined by country policy support. Policy inconsistency in applying regulation for hypoimmunogenic wheat

Table 3.7 List of bioinformatics tools

S. No.	Name of database	Main features	Primary developer/host of database	References/URL
1	Maize Genetics and Genomics Database (MaizeGDB)	Genome browser; Genome and gene annotation browser; Nested Association Mapping (NAM) founder lines (25) genome browser; qTeller: a *comparative* RNA-seqexpression platform; Metabolic pathways; etc.	United States Department of Agriculture-Agricultural Research Service (USDA-ARS)	Portwood et al. (2019) https://www.maizegdb.org/
2	MaizeMine	Gene, Gene expression, Proteins, Homology, Functions, Variations, etc.	University of Missouri	Elsik et al. (2018) http://maizemine.rnet.missouri.edu:8080/maizemine/begin.do
3	Gene Expression Omnibus (GEO)	15 microarray experiment data for maize abiotic stresses	National Center for Biotechnology Information	Clough and Barrett (2016) https://www.ncbi.nlm.nih.gov/geo/
4	ArrayExpress	15 microarray experiment data for maize abiotic stresses	European Bioinformatics Institute	Kolesnikov et al. (2015) https://www.ebi.ac.uk/arrayexpress/
5	Sequence read archive (SRA)	32 RNASeq experiment data for maize abiotic stresses	National Center for Biotechnology Information	Leinonen et al. (2010) https://www.ncbi.nlm.nih.gov/sra
6	DroughtDB	Pathway Browser for drought stress responsive genes in maize and other plants	Helmholtz Zentrum München	Alter et al. (2015) http://pgsb.helmholtzmuenchen.de/droughtdb/index.html
7	PASmiR	miRNA in abiotic stress in maize and other plants	Anhui Agricultural University	Zhang et al. (2013) http://pcsb.ahau.edu.cn:8080/PASmiR/f

(continued)

Table 3.7 (continued)

S. No.	Name of database	Main features	Primary developer/host of database	References/URL
8	ZEAMAP	A comprehensive database incorporating multiple reference genomes, annotations, comparative genomics, transcriptomes, open chromatin regions, chromatin interactions, high-quality genetic variants, phenotypes, metabolomics, genetic maps, genetic mapping loci, population structures and domestication selection signals between teosinte and maize	Huazhong Agricultural University	Gui et al. (2020) http://www.zeamap.com/
9	Panzea	Genotypicand phenotypic information for several maize populations	Cornell University	Zhao et al. (2006) https://www.panzea.org/
10	MaizeNet	Genome-scale co-functional network of maize	Yonsei University	Lee et al. (2019) http://www.inetbio.org/maizenet/
11	MaizeSNPDB	A comprehensive database for efficient retrieve and analysis of SNPs among 1210 maize lines	Henan Agricultural University	Zhou et al. 2019 https://github.com/venyao/MaizeSNPDB
12	MaizeCUBIC	Variation database for a maize synthetic population	Huazhong Agricultural University	Luo et al. (2020) http://cubicmaize.hzau.edu.cn/
13	RGPDB database	Database of Root Genes and Promoters in Maize	University of Nebraska	Moisseyev et al. (2020) http://sysbio.unl.edu/RGPDB/
14	MaizeDIG	Maize Database of Images and Genomes	Iowa State University/USDA	Cho et al. (2019) https://maizedig.maizegdb.org/

in Europe (Jouanin et al. 2018) caused a detrimental effect on the growth of niche product. To reap the benefit in term of opportunity cost they called for innovative principle of policymaking rather than the precautionary. Export-led countries like, New Zealand is a decent example to quote the need for governance and policy concise at the global level. Since the country exports, a major portion of the primary products to Europe, Asia and Australia, its investment in gene-editing technology and derived products will largely follow the trade partner's decision (Fritsche et al. 2018). Follow up of different and stringent regulation practices across the countries viz., a product-based system in Canada, mixed product/process-based system in the United States can be questionable for global food security. Shao et al. (2018) in their investigation of the relationship between new plant breeding techniques and global food security reported a reduction in the global food security as the regulations become more and more stringent and it still worsens by the political rivalry. Social acceptance is simultaneously becoming a challenge to address the fear of damage to health and the environment. Existing scientific evidence supports both pros and cons; hence, policy decisions regarding existing devices should concentrate on precautionary and responsible research.

In this context, India is also going to play a major role; not only because it produces surplus and diversified food commodities but also as one of the promising countries for technology development in the developing world. Since, new breeding techniques are expected to grow rapidly with increasing scope and applicability (Komor et al. 2016; Yin et al. 2018), consensus between society and scientific fraternity within the country and across the borders is a prime requirement to bring a workable solution by appropriate policies and governance.

3.14 Future Perspective

Maize is the most exploited cereal crop and an emerging cereal both as industrial as well as food crop in the developing world. There is a continuous demand for maize as an animal feed, which will continue to grow faster than the demand for its use as a human food, particularly in Asia. Abiotic stress is a major limiting factor to chase these demands in maize. Maize is a queen of cereals and king of genetics. Many genetic models and breeding techniques are found valid in maize improvement. Understanding on the complex traits, like abiotic stress tolerance in maize, is comparatively limited due to its complex inheritance. However, due to the availability of new breeding and genomic tools, breeding for abiotic stress tolerance in maize is becoming a reality. Further, genetic gain in maize over the decades is not much encouraging. Utilization of combination of breeding tools and techniques along with genomic designing of desired traits may yield tailor made better genotypes.

Even though, genomic designing has shown lot of promise, it has some limitations. At present, most work involving genetic engineering technology has been preliminary and hence, needs fine tuning for improving the efficiency of this

technology. Off-target incorporation is major problem in genome editing which makes hurdles in efficient editing. Incorporation of novel technology at designing efficient delivery systems and reducing the frequency of off-target editing, dissecting novel pathways and optimization of the Cas9 function may help overcome these problems. Recently, some novel cargo-vector systems have been developed which show promise for efficient delivery. Utilizing these approaches researcher can modify plants according to their expectation without negative impact on either on scientific community or on touching sociological issues. Continuous efforts are needed to overcome the above limitations to increase the versatility of genetic engineering technologies followed by targeted gene/base editing in future. In addition, public private partnership along with institutional rules and regulations certainly help the breeder to shape the maize improvement in an effective manner.

References

Abiko T, Kotula L, Shiono K, Malik AI, Colmer TD, Nakazono M (2012) Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of *Zeanicaraguensis* contribute to its waterlogging tolerance as compared with maize (*Zea mays* ssp. *mays*). Plant Cell Environ 35:1618–1630

Acquaah G (2012) Allogamy. Principles of plant genetics and breeding, 2nd edn. Wiley, Hoboken, New Jersey, pp 121–130

Alam MM, Sharmin S, Nabi Z, Mondal SI, Islam MS, Bin Nayeem S, Shoyaib M, Khan H (2010) A putative leucine-rich repeat receptor-like kinase of jute involved in stress response. Plant Mol Biol Rep 28(3):394–402

Alter S, Bader KC, Spannagl M, Wang Y, Bauer E, Schön CC (2015) Mayer KF (2015) DroughtDB: an expert-curated compilation of plant drought stress genes and their homologs in nine species. Database. 1:2015

Amara I, Capellades M, Ludevid MD, Pagès M, Goday A (2013),Enhanced water stress tolerance of transgenic maize plants over-expressing LEA Rab28 gene. J Plant Physiol 170: 864–873

Bailey-Serres J, Lee SC, Brinton E (2012) Waterproofing crops: effective flooding survival strategies. Plant Physiol 160:1698–1709

Banziger M, Cooper ME (2001) Breeding for low-input conditions and consequences for participatory plant breeding—examples from tropical maize and wheat. Euphytica 122:503–519

Banziger M, Lafitte HR (1997) Efficiency of secondary traits for improving maize for low-nitrogen target environments. Crop Sci 37:1110–1117

Banziger M, Betran FJ, Lafitte HR (1997) Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. Crop Sci 37: 1103–1109

Banziger M, Edmeades GO, Beck D, Bellon M (2000) Breeding for drought and nitrogen stress tolerance in maize. CIMMYT, Mexico DF, Mexico

Banziger M, Setimela PS, Hodson D, Vivek B (2006) Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agric Water Manage 80:212–224

Becker H (1993) Pflanzenzüchtung. Verlag Eugen Ulmer, Stuttgart

Bennet J (2001) Annex I status of breeding for tolerance of abiotic stresses and prospects for use of molecular techniques. CGIAR Technical Advisory Committee, FAO, Rome

Betrán FJ, Ribaut JM, Beck D, Gonzalez de León D (2003) Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and non-stress environments. Crop Sci 43:797–806

Bird RMK (2000) A remarkable new teosinte from Nicaragua: growth and treatment of progeny. Maize Genet Coop News 74:58–59
Bita CE, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci 4:273
Blum A (1988) Plant breeding for stress environments. CRC Press, Boca Raton, p 223
Blum A (1989) Osmotic Adjustment and growth of Barley genotypes under drought stress. Crop Sci 291:230–233
Bolanos JF, Edmeades GO (1996) The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crops Res 48:65–80
Boopathi MN (2013) Linkage mapping construction In: Boopathi (eds) Genetic mapping and marker assisted election: Basics, practice and benefits, Springer, Berlin, pp 81–108
Brugière N, Jiao S, Hantke S, Zinselmeier C, Roessler JA, Niu X, Jones RJ, Habben JE (2003) Cytokinin oxidase gene expression in maize is localized to the vasculature, and is induced by cytokinins, abscisic acid, and abiotic stress. Plant Physiol 132(3):1228–1240
Cairns JE, Prasanna BM (2018) Developing and deploying climate-resilient maize varieties in the developing world. Curr Opin Plant Biol 45:226–230. https://doi.org/10.1016/j.pbi.2018.05.004
Cairns JE, Sonder K, Zaidi PH, Verhulst N, Mahuku G, Nair BR, SK Das, B Govaerts, B Vinayan, MT Rashid Z, Noor JJ, Devi P, San Vicente F, Prasanna BM (2012) Maize production in a changing climate: impacts, adaptation and mitigation strategies. Adv Agron 114: 1–58
Cakir R (2004) Effect of water stress at different development stages on vegetative and reproductive growth of corn. Field Crop Res 89(1):1–16
Casaretto JA, El-Kereamy A, Zeng B, Stiegelmeyer SM, Chen X, Bi YM, Rothstein SJ (2016) Expression of *OsMYB55* in maize activates stress-responsive genes and enhances heat and drought tolerance. BMC Genom 17(1):1–5
Ceccarelli S, Grando S, Maatougui M, Michael M, Slash M, Haghparast R, Rahmanian M, Taheri A, Al-yassin A, Benbelkacem A, Labdi M, Mimoun H (2010) Climate change and agriculture paper: plant breeding and climate changes. J Agric Sci 148:627–637
Cho KT, Gardiner JM, Harper LC, Lawrence-Dill CJ, Friedberg I, Andorf CM (2019) MaizeDIG: maize database of images and genomes. Front Plant Sci 10:1050
Cirilo G, Dardanelli J, Balzarini M, Andrade FH, Cantarero M, Luque S, Pedrol HM (2009) Morpho-physiological traits associated with maize crop adaptations to environments differing in nitrogen availability. Field Crops Res 113:116–124
Clark RB, Alberts EE, Zobel RW, Sinclair TR, Miller MS, Kemper WD, Foy CD (1996) Eastern gamagrass (*Tripsacum dactyloides*) root penetration and chemical properties of claypan soils. In: Box JE Jr (ed) Root demographics and their efficiencies in sustainable agriculture, grasslands and forest ecosystems. Kluwer Acad Pub, Dordrecht, The Netherlands, pp 191–211
Clough E, Barrett T (2016) The gene expression omnibus database. In: Statistical genomics, Humana Press, New York, pp 93–110
Cooper M, Fukai S, Wade LJ (1999) How can breeding contribute to more productive and sustainable rainfed lowland rice systems? Field Crops Res 64:199–209
Coyne PI, Bradford JA (1985) Comparison of leaf gas exchange and water-use efficiency in two eastern gamagrass accessions. Crop Sci 25:65–75. https://doi.org/10.2135/cropsci1985.0011183X002500010018x
Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol 11:163
Dar I, Dar A, Lone ZA, Kamaluddin AA, Sofi PA, Hussan S, Dar MS, Alie W (2018) Genetic variability studies involving drought tolerance related traits in maize genotypes. J Agric Ecol Res Intl 14(2):1–13
Deinlein U, Stephan AB, Tomoaki HW, Guohua XJ, Schroeder I (2014) Plant salt-tolerance mechanisms. Trends Plant Sci 19:371–379
Edmeades GO (1993) Causes for silk delay in a lowland tropical maize population. Crop Sci 33:1029–1035
Edmeades GO (2013) Progress in achieving and delivering drought tolerance in maize—an update. ISAAA, Ithaca, NY

Edmeades GO, Bolanos J, Banziger M, Ribaut JM, White JW, Reynolds MP, Lafitte HR (1998) Improving crop yields under water deficits in the tropics. In: Chopra VL, Singh RB, Varma A (eds) Crop productivity and sustainability—shaping the future. ICSC/IBH, Oxford, UK/New Delhi, India, pp 437–451

Edmeades GO, Bolanos J, Chapman SC, Lafitte HR, Banziger M (1999) Selection improves tolerance to mid/late season drought in tropical maize populations. I. Gains in biomass, grain yield and harvest index. Crop Sci 39:1306–1315

Elsik CG, Tayal A, Unni DR, Nguyen HN, Gardiner J, Le Tourneau J, Andorf CM (2018) MaizeMine: a data mining warehouse for MaizeGDB. In: Plant and animal genome XXVI conference, January 13–17, San Diego, CA

Ersoz ES, Yu J, Buckler ES (2009) Applications of linkage disequilibrium and association mapping in maize. In: Kriz A, Larkins B (eds) Molecular genetic approaches to maize improvement. Springer, Berlin, pp 173–195

Eubanks MW (2006) A genetic bridge to utilize *Tripsacum* germplasm in maize improvement. Maydica 51(2):315–327

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A et al (2017) Crop production under drought and heat stress: plant responses and management options. Front Plant Sci 29:8 Available from: http://journal.frontiersin.org/article/10.3389/fpls.2017.01147/full

FAOSTAT (2018) Food and Agricultural Organization of the United Nations (FAO), FAO Statistical Database, http://faostat.fao.org

Farooq M, Hussain M, Wakeel A, Kadambot H, Siddique 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

Fischer RA, Byerlee D, Edmeades GO (2014) Crop yields and global food security: will yield increase continue to feed the world? ACIAR Monograph No. 158. Australian Centre for International Agricultural Research: Canberra, xxii + 634 pp

Foulkes MJ, Hawkesford MJ, Barraclough PB, Holdsworth MJ, Kerr S, Kightley S, Shewry PR (2007) Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. Field Crops Res 114:329–342

Frascaroli E, Landi P (2013) Divergent selection in a maize population for germination at low temperature in controlled environment: Study of the direct response, of the trait inheritance and of correlated responses in the field. Theor Appl Genet 126:733–746

Frey FP, Presterl T, Lecoq P, Orlik A, Stich B (2016) First steps to understand heat tolerance of temperate maize at adult stage: identification of QTL across multiple environments with connected segregating populations. Theor Appl Genet 129:945–961

Fritsche S, Poovaiah C, MacRae E, Thorlby G (2018) A New Zealand perspective on the application and regulation of gene editing. Front Plant Sci 9:1323. https://doi.org/10.3389/fpls.2018.01323

Ganther M, Vetterlein D, Heintz-Buschart A (2020) Transcriptome sequencing analysis of maize roots reveals the effects of substrate and root hair formation in a spatial context. Plant Soil. https://doi.org/10.1007/s11104-021-04921-0

Gao J, Wang S, Zhou Z, Wang S, Dong C, Mu C, Song Y, Ma P, Li C, Wang Z, He K, Han C, Chen J, Yu H, Wu J (2019) Linkage mapping and genome-wide association reveal candidate genes conferring thermotolerance of seed-set in maize. J Exp Bot 70(8):4849–4864. https://doi.org/10.1093/jxb/erz171

Gilker RE, Weil RR, Donald, El-Husseiny M (2002) Eastern gamagrass root penetration in adverse subsoil conditions. Soil Sci Soc Amer J 66. https://doi.org/10.2136/sssaj2002.0931

Gilliham M, Able JA, Roy SJ (2017) Translating knowledge in abiotic stress tolerance to breeding programs. Plant J 90:898–917

Gilmour AR, Cullis BR, Welham SJ, Thompson R (1998) (Co) Variance stricture for linear model in the analysis of plant improvement data. COMPSTAT98 proceedings in computational statistics. Physica-Verlag, Heidelberg, Germany, pp 51–64

Gitz DC, Baker JT, Stout JE, Brauer DK, Lascano RJ, Velten JP (2013) Suitability of eastern gamagrass for in situ precipitation catchment forage production in playas. Agron J 105:907–914. https://doi.org/10.2134/agronj2012.0358

Grzesiak S, Hura T, Grzesiak MT, Pienkowski S (1999) The impact of limited soil moisture and waterlogging stress conditions on morphological and anatomical root traits in maize (*Zea mays* L.) hybrids of different drought tolerance. Acta Physiol Plant 3:305–315

Gui S, Yang L, Li J, Luo J, Xu X, Yuan J, Chen L, Li W, Yang X, Wu S, Li S (2020) ZEAMAP, a comprehensive database adapted to the maize multi-omics era. iScience 23(6):101241

Habben JE, Bao X, Bate NJ, DeBruin JL, Dolan D, Hasegawa D, Helentjaris TG, Lafitte RH, Lovan N, Mo H, Reimann K (2014) Transgenic alteration of ethylene biosynthesis increases grain yield in maize under field drought stress conditions. Plant Biotechnol J 12(6):685–693

Hallauer AR, Sears JH (1969) Mass selection for yield in two varieties of maize. Crop Sci 9:47–50

Hasan MM, Rafii MY, Ismail MR, Mahmood M, Rahim HA, Alam MA, Ashkani S, Malek MA, Latif MA (2015) Marker-assisted backcrossing: a useful method for rice improvement. Biotechnol Biotechnol Equip 29:237–254

Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Intl J Mol Sci 14 (5):9643–9684. https://doi.org/10.3390/ijms14059643

He C, He Y, Liu Q, Liu T, Liu C, Wang L, Zhang J (2013) Co-expression of genes *ApGSMT2* and *ApDMT2* for glycinebetaine synthesis in maize enhances the drought tolerance of plants. Mol Breed 31(3):559–573

He Z, Zhong J, Sun X, Wang B, Terzaghi W, Dai M (2018) The Maize ABA receptors ZmPYL8, 9, and 12 facilitate plant drought resistance. Front Plant Sci 9:1–12

Hu H, Xiong L (2014) Genetic engineering and breeding of drought-resistant crops. Annu Rev Plant Biol 65:715–741

Hu G, Li Z, Yuncai Lu, Li C, Gong S, Yan S, Li G, Wang M, Ren H, Guan H, Zhang Z, Qin D, Chai M, Yu J, Li Y, Yang D, Wang T, Zhang Z (2017) Genome-wide association study identified multiple genetic loci on chilling resistance during germination in maize. Sci Rep 7:1–11

IFPRI (2003) 2025 Projections. International model for policy analysis of agriculture commodities and trade (IMPACT) special project: Global Trend in Food Security & Demand. IFPRI, Washington, DC

Iltis HH, Benz BF (2000) *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. Novon 10:382–390. https://doi.org/10.2307/3392992

IPCC (2007) Climate change 200: the physical science basis. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. IPCC Secretariat, Geneva, Switzerland

Jafar MZ, Farooq M, Cheema MA, Afzal I, Basra SMA, Wahid MA, Aziz T, Shahid M (2012) Improving the performance of wheat by seed priming under saline conditions. J Agron Crop Sci 198:38–45. https://doi.org/10.1111/j.1439-037x.2011.00487.x

Johnson SS, Geadelmann JL (1989) Influence of water stress on grain yield response to recurrent selection in maize. Crop Sci 29:558–564

Jouanin A, Boyd L, Visser RGF, Smulders MJM (2018) Development of wheat with hypoimmunogenic gluten obstructed by the gene editing policy in Europe. Front Plant Sci 9:1523. https://doi.org/10.3389/fpls.2018.01523

Kapoor M, Sveenivasan G (1988) The heat shock response of *Neurospora crassa*: stress-induced thermo tolerance in relation to peroxidase and superoxide dismutase levels. Biochem Biophys Res Commun 156(3):1097–1102

Kemper WD, Alberts EE, Foy CD, Clark RB, Ritchie JC, Zobel RW (1997) Aerenchyma, acid tolerance, and associative N fixation enhance carbon sequestration in soil. In: Lal R, Kimble JM, Follett RF, Stewart BA (eds) Management of carbon sequestration in soil. CRC Press, Boca Raton, pp 221–234

Keppler BD, Showalter AM (2010) IRX14 and IRX14-LIKE, two glycosyl transferases involved in glucuronoxylan biosynthesis and drought tolerance in Arabidopsis. Mol Plant 3(5):834–841

Khodarahmpour Z, Choukan R (2011) Genetic variation of maize (*Zea mays* L.) inbred lines in heat stress condition. Seed Plant Improv J 27(4):539–554

Kolesnikov N, Hastings E, Keays M, Melnichuk O, Tang YA, Williams E, Dylag M, Kurbatova N, Brandizi M, Burdett T, Megy K (2015) ArrayExpress update—simplifying data submissions. Nucleic Acids Res 43(D1):D1113–D1116

Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR (2016) Programmable editing of a target base in genomic DNA without double stranded DNA cleavage. Nature 533:420–424. https://doi.org/10.1038/nature17946

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E (2004) The arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO J 23(7):1647–1656

Lee T, Lee S, Yang S, Lee I (2019) MaizeNet: a co-functional network for network-assisted systems genetics in Zea mays. Plant J 99(3):571–582

Leinonen R, Sugawara H, Shumway M (2010) International nucleotide sequence database collaboration. The sequence read archive. Nucleic Acids Res 39(1):D19–21

Li B, Wei A, Song C, Li N, Zhang J (2008a) Heterologous expression of the *TsVP* gene improves the drought resistance of maize. Plant Biotechnol J 6(2):146–159

Li F, Vallabhaneni R, Yu J, Rocheford T, Wurtzel ET (2008b) The maize phytoene synthase gene family: overlapping roles for carotenogenesis in endosperm, photomorphogenesis, and thermal stress tolerance. Plant Physiol 147(3):1334–1346

Li G, Huang S, Guo X, Li Y, Yang Y, Guo Z, Kuang H, Rietman H, Bergervoet M, Vleeshouwers VG, VossenEAvd QuD, Visser RG, Jacobsen E, Vossen JH (2011a) Cloning and characterization of r3b; members of the r3 superfamily of late blight resistance genes show sequence and functional divergence. Mol Plant-Microbe Interact 24:1132–1142

Li L, Hao Z, Li X, Xie C, Li M, Zhang D, Weng J, Su Z, Liang X, Zhang S (2011b) An analysis of the polymorphisms in a gene for being involved in drought tolerance in maize. Genetica 139:479–487

Li XY, Liu ZY, Li TX (2011c) An impact test study of the flood disasters on summer corn's characters and yield. Acta Meteorol Sin 31:79–82

Li S, Yang W, Guo J, Li S, Lin J, Zhu X (2020) Changes in photosynthesis and respiratory metabolism of maize seedlings growing under low temperature stress may be regulated by arbuscular mycorrhizal fungi. Plant Physiol Biochem 154:1–10

Link W, Abdelmula AA, von Kittlitz E, Bruns S, Riemer H, Stelling D (1999) Genotypic variation for drought tolerance in Viciafaba. Plant Breed 118:477–483

Liu K, Goodman M, Muse S, Smith JS, Buckler E, Doebley J (2003) Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics 165:2117–2128

Liu Y, Qin L, Han L, Xiang Y, Zhao D (2015) Overexpression of maize SDD1 (*ZmSDD1*) improves drought resistance in *Zea mays* L. by reducing stomatal density. Plant Cell Tiss Org Cult 122(1):147–59

Liu Y, Qin L, Han L, Xiang Y, Zhao D (2015b) Over expression of maize SDD1 (ZmSDD1) improves drought resistance in *Zea mays* L. by reducing stomatal density. Plant Cell Tiss Org Cult 122:147–159. https://doi.org/10.1007/s11240-015-0757-8

Lu H, Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor Appl Genet 103:613–617

Lu Y, Li Y, Zhang J, Xiao Y, Yue Y, Duan L et al (2013) Overexpression of *Arabidopsis* molybdenum cofactor sulfurase gene confers drought tolerance in maize (*Zea mays* L.). PLoS One 8(1):e52126. https://doi.org/10.1371/journal.pone.0052126

Luo J, Wei C, Liu H, Cheng S, Xiao Y, Wang X, Yan J, Liu J (2020) (2020) MaizeCUBIC: a comprehensive variation database for a maize synthetic population. Database 1:2020

Magar M, Parajuli A, Sah B, Shrestha J, Sakha B, Koirala K, Dhital S (2019) Effect of PEG induced drought stress on germination and seedling traits of maize (*Zea mays* L.) Lines. Türk Tarım ve Doğa Bilimleri Dergisi 6(2):196–205

Mano Y, Omori F (2007) Breeding for flooding tolerant maize using "teosinte" as a germplasm resource. Plant Root 1:17–21. https://doi.org/10.3117/plantroot.1.17

Mano Y, Omori F (2008) Verification of QTL controlling root aerenchyma formation in a maize_ teosinte "*Zea nicaraguensis*" advanced backcross population. Breed Sci 58:217–223. https://doi.org/10.1270/jsbbs.58.217

Mano Y, Omori F (2013) Flooding tolerance in interspecific introgression lines containing chromosome segments from teosinte (*Zeanicaraguensis*) in maize (*Zea mays* subsp. *mays*). Ann Bot 112:1125–1139. https://doi.org/10.1093/aob/mct160

Mano YY, Muraki M, Fujimori M, Takamizo T, Kindiger B (2005) Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea mays* ssp. *huehuetenangensis*) seedlings. Euphytica 142:33–42. https://doi.org/10.1007/s10681-005-0449-2

Mano Y, Omori F, Takamizo T, Kindiger B, Bird RM, Loaisiga C et al (2007) QTL mapping of root aerenchyma formation in seedlings of a maize × rare teosinte "*Zea nicaraguensis*" cross. Plant Soil 295:103–113. https://doi.org/10.1007/s11104-007-9266-9

Mano Y, Omori F, Loaisiga CH, Bird RM (2009) QTL mapping of above-ground adventitious roots during flooding in maize x teosinte "*Zea nicaraguensis*" backcross population. Plant Root 3:3–9. https://doi.org/10.3117/plantroot.3.3

Mansouri-Far C, Modarres Sanavy SAM, Saberali SF (2010) Maize yield response to deficit irrigation during low-sensitive growth stages and nitrogen rate under semi-arid climatic conditions. Agri Water Manage 97:12–22

Melchinger AE (1999) Genetic diversity and heterosis. In: Coors JG, Pandey S (eds) The genetics and exploitation of heterosis in crops. ASA, CSSA, SSSA, Madison, WI, pp 99–118

Milivojević M, Nikolić, A Marković, K Filipović, M Vančetović, J Petrović, T Srdić J (2017) Relationship between genetic diversity and cold-tolerance of maize inbred lines. Genetika 49 (2):635–646

Mittler R (2006) Abiotic stress the field environment and stress combination. Trends Plant Sci 11:15–19

Moisseyev G, Park K, Cui A, Freitas D, Rajagopal D, Konda AR, Martin-Olenski M, Mcham M, Liu K, Du Q (2020) Schnable JC (2020) RGPDB: database of root-associated genes and promoters in maize, soybean, and sorghum. Database 1:2020

Morosini JA, Mendonça LF, Lyra DH, Galli G, Vidotti M, Fritsche-Neto R (2017) Association mapping for traits related to nitrogen use efficiency in tropical maize lines under field conditions. Plant Soil 421:453–463. https://doi.org/10.1007/s11104-017-3479-3

Morroco A, Lorenzoni C, Fracheboud Y (2005) Chilling stress in maize. Maydica 50:571–580

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nelimor C, Badu-Apraku B, Tetteh AY, N'guetta ASP (2019) Assessment of genetic diversity for drought, heat and combined drought and heat stress tolerance in early maturing maize landraces. Plants 8:518

Nelimor C, Badu-Apraku B, Tetteh AY, Garcia-Oliveira AL, N'guetta ASP (2020) Assessing the potential of extra-early maturing landraces for improving tolerance to drought, heat, and both combined stresses in maize. Agronomy 10:318. https://doi.org/10.3390/agronomy10030318

Nepolean T, Kaul J, Mukri G, Mittal S (2018) Genomics-enabled next-generation breeding approaches for developing system-specific drought tolerant hybrids in maize. Front Plant Sci 9:361. https://doi.org/10.3389/fpls.2018.00361

Nesmith DS, Ritchie JT (1992) Effects of soil water-deficits during tassel emergence on development and yield component of maize (*Zea mays*). Field Crop Res 28(3):251–256

Nguyen TX, Sticklen M (2013) Barley HVA1 gene confers drought and salt tolerance in transgenic maize (*Zea Mays* L.). Adv Crop Sci Technol 1:105

Nuccio ML, Wu J, Mowers R, Zhou HP, Meghji M, Primavesi LF, Paul MJ, Chen X, Gao Y, Haque E, Basu SS (2015) Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. Nat Biotechnol 33(8):862–869

OECD/FAO (2016) "OECD/FAO Agriculture Outlook", OECD Agriculture statistics (database). http://dx.doi.org/l0.1787/agr-outl-data-en

Omori F, Mano Y (2007) QTL mapping of root angle in F2 populations from maize 'B73' × teosinte *'Zea luxurians'*. Plant Root 1:57–65. https://doi.org/10.3117/plantroot.1.57

Partheeban C, Chandrasekhar CN, Jeyakumar P, Ravikesavan R, Gnanam R (2017) Early stage evaluation of root traits associated with drought tolerance in maize (*Zea Mays* L.) genotypes. Chem Sci Rev Lett 6(22):1301–1307

Perruc E, Charpenteau M, Ramirez BC, Jauneau A, Galaud JP, Ranjeva R, Ranty B (2004) A novel calmodulin binding protein functions as a negative regulator of osmotic stress tolerance in *Arabidopsis thaliana* seedlings. Plant J 38(3):410–420

Perumalsamy S, Bharani M, Sudha M, Nagarajan P, Arul L, Saraswathi R, Balasubramanian P, Ramalingam J (2010) Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). Plant Breed 129:400–406

Pesqueira J, Garcia M, Molina MC (2003) NaCl tolerance in maize (Zea mays ssp. mays) x Tripsacumdactyloides L. hybrid calli in regenerated plants. Span J Agric Res 1:59–63. https://doi.org/10.5424/sjar/2003012-21

Pesqueira J, García MD, Staltari S, Molina MDC (2006) NaCl effects in *Zea mays* × *Tripsacum dactyloides* hybrid calli and plants. Electr J Biotechnol 9:286–290. https://doi.org/10.2225/vol9-issue3-fulltext-29

Pietrini F, Iannelli MA, Massacci A (2002) Anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. Plant Cell Environ 25:1251–1259

Portwood JL, Woodhouse MR, Cannon EK, Gardiner JM, Harper LC, Schaeffer ML, Walsh JR, Sen TZ, Cho KT, Schott DA, Braun BL (2019) MaizeGDB, 2018 The maize multi-genome genetics and genomics database. Nucleic Acids Res 47(D1):D1146–D1154

Prasad PVV, Pisipati SR, Momcilovic I, Ristic Z (2011) Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. J Agron Crop Sci 197:430–441

Quan R, Shang M, Zhang H, Zhao Y, Zhang J (2004) Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. Plant Biotechnol J 2:477–486. https://doi.org/10.1111/j.1467-7652.2004.00093.x

Rathore TR, Warsi MZK, Lothrop JE, Singh NN (1996) Production of maize under excess soil moisture waterlogging conditions. In: 6th Asian regional maize workshop, 10–12 Feb 1996, PAU Ludhiana, pp 56–63

Ray J, Kindiger B, Dewald C, Sinclair T (1998) Preliminary survey of root aerenchyma in *Tripsacum*. Maydica 43:49–54

Ray JD, Kindiger B, Sinclair TR (1999) Introgressing root aerenchyma into maize. Maydica 44 (2):113–117

Reif JC, Xia XC, Melchinger AE, Warburton ML, Hoisington DA, Beck D, Bohn M, Frisch M (2004) Genetic diversity determined within and among CIMMYT maize populations of tropical, subtropical, and temperate germplasm by SSR markers. Crop Sci 44:326–334

Reif JC, Zhang P, Dreisigacker S, Warburton ML, van Ginkel M, Hoisington D, Bohn M, Melchinger AE (2005) Trends in genetic diversity during the history of wheat domestication and breeding. Theor Appl Genet 110:859–864

Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, Doebley J, Kresovich S, Goodman MM, Buckler ES (2001) 4th. Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proc Natl Acad Sci U S A. Sep 25;98(20):11479-84. https://doi.org/10.1073/pnas.201394398 Epub 2001 Sep 18. PMID: 11562485; PMCID: PMC58755.

Revilla P, Rodríguez VM, Ordás A, Rincent R, Charcosset A, Giauffret C, Melchinger AE, Schön CC, Bauer E, Altmann T, Brunel D, Moreno-González J, Campo L, Ouzunova M, Álvarez ÁRD, Galarreta JI, Laborde J, Malvar RA (2016) Association mapping for cold tolerance in

two large maize inbred panels. BMC Plant Biol 16:127. https://doi.org/10.1186/s12870-016-0816-2

Ribaut JM, Betran J, Monneveux P, Setter T (2009) Drought tolerance in maize. In: Bennetzen JL, Hake SC (eds) Handbook of Maize. Springer, New York, pp 311–344

Richards RA (1996) Defining selection criteria to improve yield under drought. Plant Growth Regul 20:57–166

Risser PE, Birney EC, Blocker H, May S, Parton W, Weins J (1981) The true prairie ecosystem. Hutchinson Publishing Co., Stroudsburg, PA

Rosielle AA, Hamblin J (1981) Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci 21:943–946

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA, Minx P (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326(5956):1112–1115

Schubert S, Neubert A, Schierholt A, Sumer A, Zorb C (2009) Development of salt resistant maize hybrids: the combination of physiological strategies using conventional breeding methods. Plant Sci 177:196–202. https://doi.org/10.1016/j.plantsci.2009.05.011

Semagn K, Beyene Y, Warburton ML, Tarekegne A, Mugo S, Meisel B, Sehabiague P, Prasanna BM (2013) Meta-analyses of QTL for grain yield and anthesis silking interval in 18 maize populations evaluated under water-stressed and well-watered environments. BMC Genom 14:313. https://doi.org/10.1186/1471-2164-14-313

Shao Q, Punt M, Wesseler J (2018) New plant breeding techniques under food security pressure and lobbying. Front Plant Sci 9:1324. https://doi.org/10.3389/fpls.2018.01324

Shi J, Habben JE, Archibald RL, Drummond BJ, Chamberlin MA, Williams RW, Lafitte HR, Weers BP (2015) Overexpression of *ARGOS* genes modifies plant sensitivity to ethylene, leading to improved drought tolerance in both Arabidopsis and maize. Plant Physiol 169 (1):266–282

Shou H, Bordallo P, Fan JB, Yeakley JM, Bibikova M, Sheen J, Wang K (2004) Expression of an active tobacco mitogen-activated protein kinase kinasekinase enhances freezing tolerance in transgenic maize. Proc Natl Acad Sci USA 101(9):3298–3303

Strigens A, Freitag NM, Gilbert X, Grieder C, Riedelsheimer C, Schrag TA, Messmer R, Melchinger AE (2013) Association mapping for chilling tolerance in elite flint and dent maize inbred lines evaluated in growth chamber and field experiments. Plant Cell Environ 36:1871–1887

Su Z, Li X, Hao Z, Xie C, Li M, Weng J, Zhang D, Liang X, Wang Z, Gao J, Zhang S (2011) Association analysis of the *nced* and *rab28* genes with phenotypic traits under water stress in maize. Plant Mol Biol Rep 29:714–722

Sulmon C, Gouesbet G, Ramel F, Cabello-Hurtado F, Penno C, Bechtold N, Couee I, El Amrani A (2011) Carbon dynamics, development and stress responses in arabidopsis: involvement of the APL4 subunit of ADP-glucose pyrophosphorylase (starch synthesis). PLoS ONE 6(11):

Suresh S, Malathi D (2013) Gene pyramiding for biotic stress tolerance in crop plants. Wkly Sci Res J 1:1–14

Tandzi LN, Bradley G, Mutengwa C (2019) Morphological responses of maize to drought, heat and combined stresses at seedling stage. J Biol Sci 19(1):7–16

Tarter JA, Goodman MM, Holland JB (2004) Recovery of exotic alleles in semi-exotic maize inbreds derived from crosses between Latin American accessions and a temperate line. Theor Appl Genet 109:609–617

Thornton PK, Jones PG, Alagarswamy G, Andersen J (2009) Spatial variation of crop yield response to climate change in East Africa. Glob Environ Change 19(1):54–65

To JP, Haberer G, Ferreira FJ, Deruere J, Mason MG, Schaller GE, Alonso JM, Ecker JR, Kieber JJ (2004) Type-A arabidopsis response regulators are partially redundant negative regulators of cytokinin signaling. Plant Cell 16(3):658–671

Vigouroux Y, Mitchell S, Matsuoka Y, Hamblin M, Kresovich S, Smith JSC, Jaqueth J, Smith OS, Doebley J (2005) An analysis of genetic diversity across the maize genome using microsatellites. Genetics 169:1617–1630

Vuylsteke MJR (1999) Genetic analysis of maize by using the AFLP method. PhD thesis, University of Wageningen, Netherlands

Vuylsteke M, Kuiper M, Stam P (2000) Chromosomal regions involved in hybrid performance and heterosis: their AFLPbased identification and practical use in prediction models. Heredity 85:208–218

Wang H, Qin F (2017) Genome-wide association study reveals natural variations contributing to drought resistance in crops. Front Plant Sci 8:1110

Wang W, Vinocur B, Altman A (2007) Plant responses to drought, salinity and extreme temperatures towards genetic engineering for stress tolerance. Planta 218:1–14

Wang CR, Yang AF, Yue GD, Gao Q, Yin HY, Zhang JR (2008) Enhanced expression of phospholipase C 1 (*ZmPLC1*) improves drought tolerance in transgenic maize. Planta 227 (5):1127–1140

Wang AY, Li Y, Zhang CQ (2012) QTL mapping for stay-green in maize (*Zea mays*). Can J Plant Sci 92:249–256

Wang X, Wang H, Liu S, Ferjani A, Li J, Yan J, Yang X, Qin F (2016) Genetic variation in ZmVPP1 contributes to drought tolerance in maize seedlings. Nat Genet 48:1233–1241. https://doi.org/10.1038/ng.3636

Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J 4(3):162–176

Warburton ML, Xia X, Crossa J, Franco J, Melchinger AE, Frisch M, Bohn M, Hoisington D (2002) Genetic characterization of CIMMYT inbred maize lines and open pollinated populations using large scale fingerprinting methods. Crop Sci 42:1832–1840

Warburton ML, Reif JC, Frisch M, Bohn M, Bedoya C, Xia XC, Crossa J, Franco J, Hoisington D, Pixley K, Taba S, Melchinger AE (2008) Genetic diversity in CIMMYT nontemperate maize germplasm: landraces, open pollinated varieties, and inbred lines. Crop Sci 48:617–624

Wei A, He C, Li B, Li N, Zhang J (2011) The pyramid of transgenes *TsVP* and *BetA* effectively enhances the drought tolerance of maize plants. Plant Biotechnol J 9(2):216–229

Xia XC, Reif JC, Hoisington DA, Melchinger AE, Frisch M, Warburton ML (2004) Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: I. Lowland tropical maize. Crop Sci 44:2230–2237

Xia XC, Reif JC, Melchinger AE, Frisch M, Hoisington DA, Beck D, Warburton ML (2005) Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: II. Subtropical, tropical mid-altitude, and highland maize inbred lines and their relationships with elite US and European maize. Crop Sci 45:2573–2582

Yadav OP, Weltzien-Rattunde E, Bidinger FR (2003) Genetic variation in drought response of landraces of pearl millet (*Pennisetum glaucum* (L.) R. Br.). Indian J Genet 63:37–40

Yang L, Fu FL, Deng LQ, Zhou SF, Yong TM, Li WC (2012) Cloning and characterization of functional keratin-associated protein 5–4 gene in maize. Afr J Biotechnol 11(29):7417–7423

Yi Q, Malvar RA, Álvarez-Iglesias L, Ordás B, Revilla P (2020) Dissecting the genetics of cold tolerance in a multi parental maize population. Theor Appl Genet 133(2):503–516. https://doi.org/10.1007/s00122-019-03482-2

Yin G, Gu J, Zhang F, Hao L, Cong P, Liu Z (2014) Maize yield response to water supply and fertilizer input in a semi-arid environment of northeast china. PLoS ONE 9:

Yin Y, Wang Q, Xiao L, Wang F, Song Z, Zhou C et al (2018) Advances in the engineering of the gene editing enzymes and the genomes: understanding and handling the off-target effects of CRISPR/Cas9. J Biomed Nanotechnol 14:456–476. https://doi.org/10.1166/jbn.2018.2537

Yuan Y, Cairns JE, Babu R, Gowda M, Makumbi D, Magorokosho C, Zhang A, Liu Y, Wang N, Hao Z, Vicente FS, Olsen MS, Prasanna BM, Lu Y, Zhang X (2019) Genome-wide association mapping and genomic prediction analyses reveal the genetic architecture of grain yield and flowering time under drought and heat stress conditions in maize. Front Plant Sci 9:1919. pmid:30761177

Zhang S, Yue Y, Sheng L, Wu Y, Fan G, Li A, Hu X, Shang GM, Wei C (2013) PASmiR: a literature-curated database for miRNA molecular regulation in plant response to abiotic stress. BMC Plant Biol 13(1):33

Zhang J, Yu H, Zhang Y, Wang Y, Li M, Zhang J, Duan L, Zhang M, Li Z (2016) Increased abscisic acid levels in transgenic maize overexpressing *AtLOS5* mediated root ion fluxes and leaf water status under salt stress. J Exp Bot 67(5):1339–1355

Zhao W, Canaran P, Jurkuta R, Fulton T, Glaubitz J, Buckler E, Doebley J, Gaut B, Goodman M, Holland J, Kresovich S (2006) Panzea: a database and resource for molecular and functional diversity in the maize genome. Nucleic Acids Res 34(1):D752–D757

Zheng HJ, Wu AZ Zheng CC, Wang YF, Cai R, Shen XF, Xu RR, Liu P, Kong LJ, Dong ST (2009) QTL mapping of maize (Zea mays) stay-green traits and their relationship to yield. Plant Breed 128:54–62

Zhou W, Wang L, Zheng W, Yao W (2019) MaizeSNPDB: a comprehensive database for efficient retrieve and analysis of SNPs among 1210 maize lines. Comput Struct Biotechnol J17:1377–1383

Zielinski RE (1998) Calmodulin and calmodulin-binding proteins in plants. Annu Rev Plant Biol 49(1):697–725

Chapter 4
Genetic Diversity for Barley Adaptation to Stressful Environments

Agostino Fricano, Raffaella Battaglia, Erica Mica, Alessandro Tondelli, Cristina Crosatti, Davide Guerra, and Luigi Cattivelli

Abstract Breeding for abiotic stress tolerance means employing genetics to increase the capacity of a crop to withstand physical stress factors without penalizing yield. Over 50 years of studies on barley stress tolerance have contributed to the description of the genetic diversity and of the molecular components underlying the stress response as well as to the identification of the main genes and loci involved in stress resistance. This knowledge has been translated into new varieties with improved stress tolerance, although the new challenges imposed by climate change still require extensive work to select barley adapted to the new climatic scenarios and new breeding strategy is needed. This chapter reviews the fundamental knowledge acquired on barley tolerance and adaptation to abiotic stresses and describes how the new tools available for breeding, namely Genomics Selection and Genome Editing, can be applied to increase the rate of genetic progress in breeding for stress-prone environments and to expand genetic diversity introgressing new resistant traits in barley cultivars.

Keywords Barley · Abiotic stress tolerance · Adaptation to environment · Drought · Cold · Salinity · Flowering time · Genomic selection · Genome editing

4.1 Introduction

Barley (*Hordeum vulgare*), the fourth most important cereal, is a major crop grown worldwide on about 50 million ha with a global annual production between 140 and 150 million tons. Europe is the most important barley producer with 80–90 million

A. Fricano · R. Battaglia · E. Mica · A. Tondelli · C. Crosatti · D. Guerra · L. Cattivelli (✉)
CREA Research Centre for Genomics and Bioinformatics, Via San Protaso, 302, 29017 Fiorenzuola D'Arda, Italy
e-mail: luigi.cattivelli@crea.gov.it

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tons harvested every year (http://www.fao.org/faostat/en/). The major destination of barley is for feed, but malt either for beer or whisky production is an important and traditional use and it is far more valuable. Barley is also employed as human food and thanks to its high seed β-glucan content can contribute to reducing blood cholesterol.

Barley has been domesticated from its wild progenitor (*H. vulgare* ssp. *spontaneum*) around 10,000 BC in the Fertile Crescent region, an event associated to the mutation in the gene controlling spike shattering (Haas et al. 2018). Two linked dominant complementary genes (*Btr1* and *Btr2*) control rachis shattering and independent mutations in each of them lead to the non-shattering form (non-brittle rachis). Moving from its center of diversification, barley is now cultivated over a wide range of latitudes (from Africa to Iceland), altitudes (from sea level up to 4500 m in Tibet) and conditions (up to extreme environments such as the limit of deserts) where other cereal crops fail. The ability of barley to grow in so many different environments reflects the overall stress tolerance capacity of this crop and a large diversity for the mechanisms controlling flowering time in response to vernalization, photoperiod and temperature (Drosse et al. 2014). Indeed, barley is considered more drought tolerant than wheat and has an extremely flexible life cycle (from as short as 90 days up to 9–10 months).

The significant stress resilience capacity of barley generally is based on adaptive mechanisms elicited when the plant is exposed to non-lethal unfavourable events. A network of hormones and transcription factors controls the response to stress and supports the expression of large sets of genes which, in turn, determine many physiological and metabolic modifications (Zhu 2016). The level of stress tolerance achieved through these adaptive mechanisms is dependent on the specific genotype and, to some extent, on plant's growth stage (Cattivelli et al. 2008; Tondelli et al. 2020).

In most barley fields, this crop experiences a large deviation from optimal environmental requirements, due to limiting factors (stresses) that do not allow to express its full agronomic value. An abiotic stress can result from the shortage of an essential resource (i.e. mineral nutrients, water), from the excess of a toxic substance (salinity) or from climatic extremes (cold or heat temperatures, wind-lodging). Occurrence, severity, timing, and duration of stresses vary from location to location and from year to year. Furthermore, an abiotic stress seldom occurs alone, and the plants often face growing conditions characterized by a combination of different physical stresses (Cattivelli et al. 2008).

Barley yield is limited by the extent of abiotic (and biotic) stresses and their impact is expected to increase in the future. The warming of the climate system is unequivocal and there are many evidence of increasing in global average temperatures, melting of ice and rising of the sea level, IPCC 2013). Consequently, it is expected that the frequency and severity of extreme temperatures and drought events will increase and will negatively impact crop yield. A recent work suggests that climate changes may cause a substantial reduction of barley yield estimated between 3 and 17%, on worldwide basis, depending on the severity of the future climatic conditions (Xie et al. 2018).

Drought, the main factor limiting crop production, is often associated with high temperatures that impose an additional level of stress to plants. Irrigation can alleviate drought, but it contributes to increasing soil salinization and an excessive amount of soluble salts, mainly sodium chloride, in soils is becoming a mounting emergence worldwide (Hanin et al. 2016). The expected climatic change will also modify the annual temperature profile (less frost during winter, more heat stress during summer (IPCC 2013), which implies a consequent variation in the sowing date, growth habit or/and heading time (Marcinkowski and Piniewski 2018).

4.2 Barley Adaptation to Environment

The synchronization of flowering time to the most favourable environmental and seasonal conditions is crucial for the plant to maximize yield formation, i.e., to set the maximum number of seeds, still being able to fill them (Wiegmann et al. 2019). Internal and external signalling pathways centered on major genes that act as integrator hubs finely regulate the floral transition. The major components controlling barley flowering time, vernalization requirement, photoperiod response and earliness *per se*, are well known and most of the corresponding genes have been described and isolated (McKim et al. 2018 and references therein). Here we summarize the main findings.

- **Vernalization requirement**: a period of exposure to low temperatures is necessary for flowering induction in the wild relative of cultivated barley, *H. vulgare* spp. *spontaneum*, and in accessions with a winter growth habit. This trait is controlled by three loci (i.e., *Vrn-H1*, *Vrn-H2* and *Vrn-H3*), and mutations at *Vrn-H1* and *Vrn-H2* lead to the spring and facultative growth habits in most of the domesticated barley genotypes. *Vrn-H1* is induced during exposure to low temperature, then, after vernalization, the stably high expression level of *Vrn-H1* in meristematic tissues promotes the transition from vegetative to reproductive phase (Xu and Chong 2018). The memory of vernalization is due to epigenetic modifications in both promoter and first intron, while the occurrence of a deletion in the 10 kb first intron of *Vrn-H1* prevents the epigenetic control of gene expression and promotes flowering without vernalization (spring grow habit) (Oliver et al. 2013). In view of climate change, facultative barley cultivars are getting an increase interest due of their frost resistance without vernalization requirement, and their larger flexibility in planting date (Muñoz-Amatriaín et al. 2020).
- **Photoperiod sensitivity**: sensing the increase of day length in spring is crucial for barley to ensure that flower development occurs when there is low risk of frost events. *Ppd-H1* is the main sensor of long day, acting by inducing the expression of the florigen *HvFT1* (*VRN-H3*), thus integrating the vernalization

and photoperiod pathways. Mutations at *Ppd-H1* lead to less photoperiod-sensitive genotypes, better adapted to higher latitude because of their delayed flowering and better exploitation of water and other resources in spring-sown barley (Turner et al. 2005; von Korff et al. 2006). Recently, *Ppd-H1* has also been related to developmental plasticity in response to drought (Gol et al. 2021). Recessive alleles at a second photoperiod response locus (*Ppd-H2*) are often associated to the winter growth habit to delay flowering under short day conditions in the winter.

- **Earliness per se or early maturity**: genes that induce floral development independently from photoperiod are in most cases related to the circadian clock (*Eam5, Eam8, Eam10*) (Hu et al. 2019; Pham et al. 2020). Early flowering in loss of function mutants occurs through induction of *HvPRR37* and/or *HvFT1* expression (Campoli et al. 2012).

Haplotype variation in the master switches controlling flowering time allows the fine tuning of flowering transition that, in turn, explains the agroecological adaptation and allows the adaptation to specific environmental cues and possibly to future climate change Casas et al. (2011) observed a non-random latitudinal distribution of *HvFT1* alleles in barley landraces collected throughout Spain, with genotypes individual carrying specific haplotype at the promoter associated with early flowering prevailing in the southern part of the country to escape terminal water stress. More recently, the pattern of allelic variation observed for 19 flowering genes in a collection of 267 georeferenced wild barleys and landraces was associated with the site of origin and intrinsic environmental variables (Russell et al. 2016). Among the loci considered, *HvCEN/EPS2*, one of the main loci differentiating winter and spring barley gene pools (Comadran et al. 2012), showed the strongest association with latitude. Robust geographical structuring was detected also for multiple-gene haplotypes and such allelic combinations matched life history traits (i.e., heading date and plant height) evaluated in common garden experiments at different locations (Russell et al. 2016). Similarly, genotype-by-environment modelling on a diverse panel of 371 domesticated barley accessions evaluated for heading date across contrasting environments indicated a consistent effect of *HvCEN* in anticipating flowering, while contrasting responses of *Ppd-H1* alleles were observed in spring-sown and winter-sown trials (Bustos-Korts et al. 2019).

Other than focusing on known genes, genome-wide molecular variants from georeferenced accessions can be combined with bioclimatic parameters of the sampling sites to identify markers statistically associated with environmental constraints. By comparing wild barley populations separated by the Zagros Mountains, significant associations with both temperature and precipitation variables were detected for single nucleotide polymorphisms (SNPs) on chromosomes 2H and 5H (Fang et al. 2014). Similar environmental association analyses pointed at specific genomic regions involved in adaptations of Spanish barley landraces to agroclimatic features related to temperature and water availability (Contreras-Moreira et al. 2019). Once adaptive traits and/or genomic regions are associated to environmental

parameters, genebanks can be more easily queried for georeferenced accessions with specific features. This informed exploitation of the genetic resources can support the identification of new allele and the development of climate-resilient cultivars tailored to specific environments.

4.3 Genetics and Physiology of Cold Tolerance

The abilityof barley and wheat to survive over winter (winter-hardiness) is related to their level of frost tolerance. Frost tolerance is a major component of the mechanisms that allow plants to survive during winter and to synchronize their life cycle with the seasonal cycle. Winter hardiness is a broader concept that express the ability of a plant to cope with all liming factors associated with winter (i.e., frost, pathogens specifically adapted to low temperature, anoxia) (Cattivelli 2011).

Different methods are used for phenotyping barley for frost tolerance (Cattivelli and Crosatti 2020). While winter hardiness is tested in the field during vegetative growth (Fig. 4.1), frost tolerance is usually evaluated under controlled conditions throught the measure of frost-induced damage and/or plant survival after exposure to freezing temperatures. Fast and reliable experimental systems, like those based on chlorophyll fluorescence (Rizza et al. 2001), are used for precise monitoring of frost tolerance. Any damage to cellular integrity, i.e. the damaged induced by freezing, leads to a decrease in the maximum quantum yield of the photosystem II (PSII). This parameter can be easily assessed by measuring the ratio of variable (Fv) to maximal (Fm) chlorophyll fluorescence in the dark-adapted state (Fv/Fm parameter; Rizza et al. 2001).

Maximum frost tolerance is depending on a coordinated genetic network induced upon exposure to low non-freezing temperatures, a process known as cold acclimation or hardening (Rizza et al. 1994). Low temperature induces a general metabolic switch involving alterations in membrane composition, increased activity

Fig. 4.1 A winter nursery for field assessment of frost tolerance (left) and a plant showing leaves damaged by winter frost (right)

of enzymes for sucrose synthesis, ROS scavenging and xanthophyll cycle, enhanced capacity for energy dependent fluorescence quenching, accumulation of osmolytes (e.g., proline, glycinebetaine), soluble sugars, abscisic acid, induction of Cold-Regulated (*COR*) genes and accumulation of the corresponding COR proteins (Cattivelli and Bartels 1990; Murelli et al. 1995; Crosatti et al. 1995; Ensminger et al. 2006).

The molecular response to cold is trigged by the low temperature-dependent activation of *ICE1*, a MYC-like basic helix-loop-helix transcription factor (Skinner et al.2006; Badawi et al. 2008) that induces the expression of the *C-repeat binding factor* (*CBF*) genes, whose products activate a the *COR* genes leading to the accumulation of COR proteins with a direct role in protecting plant cells from frost damage (Dal Bosco et al. 2003; Skinner et al. 2005; Tondelli et al. 2011). A faster accumulation rate and a higher levels of *COR* genes and proteins have been found in comparisons between frost tolerant and frost sensitive barley cultivars (Crosatti et al. 1995) and transgenic barley overexpressing *CBF* genes of wheat (*TaCBF14* and *TaCBF15*) showed enhanced cold acclimation and frost tolerance (Soltesz et al. 2013). More recently the analyses of molecular response to low temperatures have highlighted the role of miRNAs that may regulate, in turn, different key transcription factors to coordinately fine tune the molecular response to cold. For instance, miR159 play an important role in cold, salt, heat and drought stress responses in wheat (Song et al. 2017), mediating the post-transcriptional regulation of MYB transcription factors.

Extensive genetic analyses in barley have identified two main *Frost Resistance* (*Fr*) loci mapped 30 cM apart on the long arm of the chromosome 5H and accounting for a large proportion of the observed phenotypic diversity (Francia et al. 2004; Skinner et al. 2006; von Zitzewitz et al. 2011; Tondelli et al. 2014). The *Fr-H2* locus co-segregates with a cluster of *CBF* genes (Francia et al. 2007; Tondelli et al. 2011) providing a direct link between a transcription factor with a major role in cold acclimation and the genetic diversity for frost tolerance. The barley *CBF* locus is a gene cluster encompassing more than 11 members with some of them subject to copy number variations. Although there are many evidence supporting the role of the *CBF* locus in frost tolerance, a relevant open question concerns the identification of the specific *CBF* sequence responsible for the extensive genetic variation observed in barley. Some works suggested that variations in the number of copies of the *HvCBF2-HvCBF4* region might be the causal functional polymorphism underlying frost tolerance (Knox et al. 2010; Francia et al. 2016). Nevertheless, a candidate gene association mapping study identified allelic variation at *HvCBF14* has the most related polymorphisms tied to frost tolerance (Fricano et al. 2009). *Fr-H1* locus represents a pleiotropic effect of the major vernalization gene *Vrn-H1* (Yan et al. 2003; von Zitzewitz et al. 2005). Finally, a further *Fr* locus, *Fr-H3* on chromosome 1HS, was found to give a positive allelic contribution to frost acclimation particularly in facultative barley genotypes (Fisk et al. 2013; Munoz-Amatrian et al. 2020).

Temperatures that induce cold acclimation also satisfy the vernalization requirement of winter barley, allowing the switch from vegetative to the reproductive growth and suggesting an interconnection between the two processes. Coherently, levels of frost tolerance decrease after vernalization and the extent of *COR* gene expression is influenced by allelic variation at *Vrn-H1* with increased levels of *Vrn-H1* mRNAs associated to reduced *CBF* expression (Stockinger et al. 2007).

Spring barleys are, on average, less frost tolerant than winter of facultative cultivars, nevertheless even spring genotypes activate a cold acclimation process in response to low temperature, and a significant genetic diversity has been observed for frost tolerance within spring germplasm, mainly associated to allelic variation at the *Fr-H2* locus (Tondelli et al. 2014).

Overall, the genetic data available for barley point out that frost tolerance is a quantitative trait dominated by two linked loci, a condition that makes genetic improvement for this trait a relatively easy target, within the limits of the available genetic diversity. Notably, a significant increase in frost tolerance was found in moderns *vs*. old spring barley genotypes (Tondelli et al. 2014). Global warming is causing more mild winters and larger fluctuation of winter temperatures (Bellard et al. 2012); this climatic scenario will shift the cultivation of winter barley toward Northern latitudes and support the use of facultative or spring cultivars during winter sowing in warmer regions (Muñoz-Amatriaín et al. 2020).

4.4 Genetics and Physiology of Drought Tolerance

4.4.1 The Complex Nature of Drought

Drought refers to a condition of water shortage, which induces morphological, biochemical, physiological, and molecular changes in crops and leads to final yield loss. Besides, drought often occurs simultaneously with other adverse weathering, mainly high temperatures, which impose further stress to the plant (Lawas et al. 2018). There are three generally accepted strategies that a crop can adopt to overcome drought, namely escape, avoidance and tolerance (Chaves et al. 2003) (Fig. 4.2). Drought escape refers to the ability of a plant to ensure a successful reproduction by completing its life cycle before the onset of the stress and it is positively associated with early flowering (see Sect. 4.2: Barley Adaptation to Environment). Conversely, drought avoidance refers to the ability of the plant to sustain growth and maintain a high tissue water content despite reduced water in the soil (Blum 2005) and it is mainly associated with small or closed stomata, reduced photosynthesis and a slow growth rate (Shavrukov et al. 2017). Finally, when a plant can withstand dehydration and sustain growth despite the occurrence of drought through osmotic adjustment and production of protectants molecules, then it is referred to as drought tolerance (Tardieu et al. 2018).

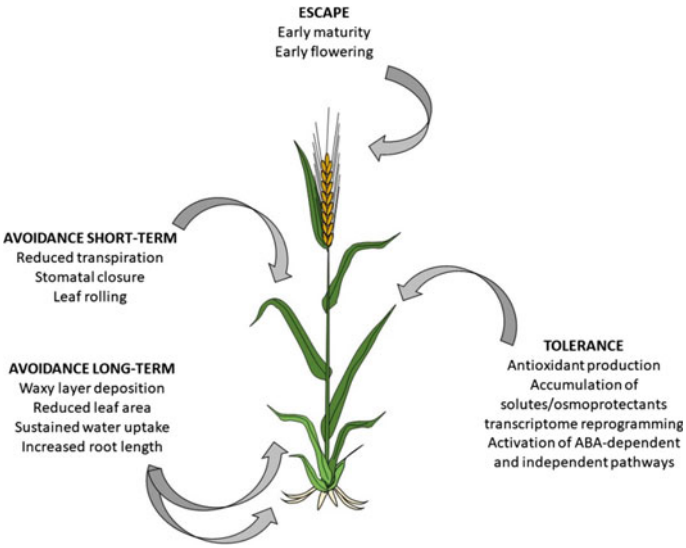

Fig. 4.2 Plant response to drought stress encompass three main strategies: drought escape, drought avoidance and drought tolerance. According to the time span, the plant response can include short-term and long-term reactions being the former mainly at physiological level and the latter at morphological level

From a physiological point of view, a plant experiences drought when evapotranspiration exceeds water uptake. In these conditions, a low leaf water potential leads to a decrease in leaf turgor, stomatal conductance and photosynthesis, thus limiting plant growth and yield. Depending on the timing, duration and intensity of the stress, the water deficit has different effects on the cellular processes, triggers different response mechanisms (e.g., the accumulation of osmolytes to maintain cell turgor pressure, a process known as osmotic adjustment, Moinuddin et al. 2005) and limits yield through different mechanisms (Tardieu et al. 2018; Kumar et al. 2018).

On the bases of the mechanisms that plants adopt to reduce transpiration in response to drought, crops have been classified in isohydric (water savers) like maize, pea and lupin, and anisohydric (water spenders) like wheat and barley. Nevertheless, a genetic diversity for this trait exists also within species, and barley genotypes with a different level of isohydric or anisohydric behavior are known. While isohydric genotypes exert a tight control of preventive stomata closure to maintain leaf water potential, anisohydric plants do not (Tardieu and Simonneau 1998; Comstock 2002). Indeed, isohydric plants adopt a safer strategy at the expense of biomass production, while anisohydric plants aim to maintain an active growth at high risk of hydraulic failure (i.e., xylem embolism). Most of the water loss from plants occurs via transpiration through stomata, and experimental evidence have demonstrated that it is possible to improve drought tolerance (and water

use efficiency) by reducing the density of stomata on leaves either with mutations or biotechnological approaches (Yoo et al. 2010).

When drought lasts over time it also induces morphological changes of plant organs and tissues like deeper root system or modifications of the aerial parts of the plant. An increase in root/shoot ratio was frequently observed in drought-stressed plants (Poorter et al. 2012). Leaves undergo modifications to decrease the evaporating area, following the reduction of cell turgor, leaf rolling occurs, and deposition of epicuticular waxes helps to reduce non-stomatal transpiration (Bodner et al. 2015). Finally, if the severity or the duration of the stress increases then leaves start to wither leading to senescence and remobilization of photosynthates to other organs (Munné-Bosch and Alegre 2004).

4.4.2 *Barley Loci for Drought Tolerance*

A pre-requisite for any breeding program is the availability of genetic variation, nevertheless there are indications that in the last decades the genetic diversity of barley elite cultivars has been narrowed due to a strong selection for a high yield under favourable conditions (Kalladan et al. 2013). A large panel of genetic diversity, reliable traits to measure, and accurate phenotyping methods are the key requirements for the identification of drought-tolerant genotypes. To evaluate the best combination of traits and assay for selecting drought-tolerant genotypes, a worldwide collection of 237 cultivated and 190 wild barleys has been tested in different drought-prone environments at different growth stages (Cai et al. 2020). A high degree of genetic variability has been observed both in cultivated and wild barley although the latter showed better performance under drought stress.

The cultivation area of barley and its wild progenitor (*H. vulgare* spp. *spontaneum*) extends over regions where drought can occur anytime during life cycle indicating a wide adaptation potential of the crop to harsh environments (Araus et al. 2002; Turner 2004; Xie et al. 2018). The use of crop wild relatives as a source of useful variation it is making a comeback (Feuillet et al. 2008) and the diversity harbored by wild barley for drought tolerance is well known since many years (Nevo et al. 1981, 1983, 1986). For instance, when a population of wild barley from a region in the north of the sea of Galilee was characterized for the response to drought a large phenotypic variation was found for traits related to yield, fertility and morphology both in well-watered and stressed conditions (Ivandic et al. 2000). The role of wild barley as a source of diversity for drought tolerance has been also tested in a panel of 166 Tibetan wild barley accessions phenotyped in either hydroponic or pot experiments. Large genotypic and phenotypic variations for root morphology, biomass accumulation, antioxidant activity (catalase and peroxidase) and soluble protein content in leaves have been observed and 91 trait-marker associations were identified for physiological and morphological traits (Zhang et al. 2019).

The growth stage at which stress occurs is another key concept to consider when screening for diversity. In barley, water deprivation during the early growth phase mainly affects the plant biomass and spike fertility, while terminal stress affects grain filling, thus it is crucial to screen for diversity at each stage during the crop life cycle (Cattivelli et al. 2008). For instance, in a study aimed at identifying drought-tolerant genotypes, 47 wild barley introgression lines from the S42IL population (Schmalenbach et al. 2008) were phenotyped at the juvenile stage in pot experiments for fourteen traits related to drought tolerance and 44 quantitative trait loci (QTLs) were identified (Honsdorf et al. 2014). Interestingly, this study found a positive effect of exotic alleles for growth rate under stress conditions as well as a negative effect of the exotic allele on water use efficiency. The same wild barley introgression lines population was phenotyped in greenhouse to study genetic diversity for tolerance to terminal drought scoring traits such as days to heading, plant height, aboveground biomass and yield-components. Forty QTLs were detected of which the most promising candidates are those for grain numbers per ear and thousand-grain weight (Honsdorf et al. 2017). Recently a QTL on chromosome 4H responsible of an increase in biomass of 10–17% contributed by a wild barley accession has been identified using a nested association mapping (NAM) population of 1420 BC1S3 lines (Pham et al. 2019).

The large dataset of QTLs reported in the literature for barley have been summarized in a meta-QTL analysis by Zhang et al. (2017) and 72 major QTLs were reported for drought tolerance related traits such as: germination rate, early growth vigour, wilting, recovery rate, yield components, root morphology, water relations, water-soluble carbohydrates, morphological traits and chlorophyll fluorescence.

4.4.3 Candidate Genes for Drought Tolerance

At the molecular level drought induces the expression of many genes (for barley see Cantalapiedra et al. 2017) regulated through complex transcriptional networks mediated by dehydration responsive element-binding (*DREB*)-type transcription factors (Kumar et al. 2018). *DREB* transcription factors triggers the expression of many drought-responsive genes, including the *LEA* (also known as *Dehydrin*) gene family (Choi et al. 1999) and the genes for the biosynthesis of osmolytes, and have been suggested as biotechnological target for drought improvement also in barley (Morran et al. 2011). It is remarkable that the overexpression of barley *HVA1*, a Group 3 LEA gene, was able to confer better growth and higher water use efficiency to transgenic wheat plants (Sivamani et al. 2000).

HVA1 is activated by *HvDRF1*, an AP2/ERF class of transcription factors, involved in ABA dependent drought response. Under stress condition, ABA promotes alternative splicing of the transcript, producing an active form of *HvDRF1*, that is able to bind *HVA1* promoter (Xue and Loveridge 2004). Additionally, post transcriptional regulation mediated by miRNAs has been deeply investigated in barley plants under drought stress, the most interesting example is the increased

water use efficiency and recovery ability after drought achieved by over-expressing *Hv-miR827* under a drought inducible promoter (Ferdous et al. 2017). Even though targets have not been demonstrated for this miRNA in barley, it is supposed that miR827 is involved in maintaining photosynthetic activity and assimilation under unfavourable conditions.

Higher proline accumulation has been related to higher tissue water status and efficiency of photochemistry under drought conditions. Muzammil et al. (2018) have demonstrated that an ancestral allele of the *pyrroline-5-carboxylate synthase* (*P5cs1*) gene from a wild barley accession is a major responsible of a higher drought-inducible proline accumulation under water shortage. Genetic analyses suggested that the causal mutations controlling drought-inducible proline accumulation might lie in the promoter of *P5cs1* where variants within putative ABA responsive element motifs were found in cultivated versus wild barley.

The epidermal patterning factor (EPF) regulates the frequency of stomatal development and when the barley orthologous, *HvEPF1*, is overexpressed the plants showed a reduction in stomatal density and in leaf gas exchange, an enhanced water use efficiency and no significant reduction in grain yield. These results demonstrate the potential of manipulating stomatal density and represent a potential strategy for the optimization of cereal crop, although a field evaluation is still missing (Hughes et al. 2017).

A QTL for leaf wilting was co-localized with the locus for 9-cis-epoxycarotenoid dioxygenase 2 (*HvNCED2*, chromosome 5H), a gene involved in the synthesis of abscisic acid (Fan et al. 2015). Furthermore, a QTL on chromosome 5H associated with the dry weight of roots in response to drought (Reinert et al. 2016) co-segregated with two paralogous genes, *HvCBF10B* and *HvCBF10A*, proposed as candidates due to the presence a 37 amino acid deletion and substitution mutations in the conserved domain of two genes, respectively.

Root system architecture is a fundamental component of drought tolerance and deeper roots have been often associated to a superior drought resistance. A steep, cheap, and deep root systems was observed in Australian barley varieties from drought-prone environments carrying a spring allele at the major vernalization-requirement gene, *HvVRN1* (Voss-Fels et al. 2018). In wheat and barley lines with different root architecture, the orthologous genes to the rice *DRO1* (*Deep Rooting 1*) which contribute to increasing the yield under drought conditions, have been identified and variation in the promoter regions have been associated to the root development (Uga et al. 2013, Ashraf et al. 2019).

4.4.4 The Additional Effect of Heat Stress

Because of lower plant evapotranspiration and high air temperature observed under prolonged periods of water scarcity, under field conditions plants frequently experience drought in combination with heat stress (Lawas et al. 2018). Heat stress

per se causes a reduction of the photosynthetic activity, accelerates grain filling and anticipates plant senescence. This decline limits the translocation of the assimilates into the kernels leading to a reduced grain weight (Wardlaw and Wrigley 1994). The catalytic activity of enzymes is often reduced as soon as the temperature exceeds the optimal value. In seeds, high temperatures have a negative impact on many enzymes of the starch biosynthetic pathway (ADP-glucose pyrophosphorylase, branching enzyme, granule bound starch synthase, soluble starch synthase), that, together with the limited supply of carbon from photosynthetic tissues, results in a reduced accumulation of starch during heat stress (Wallwork et al. 1998). At cellular level, heat stress triggers multiple signal transduction pathways that lead to an enhanced thermotolerance. The *Heat Shock Factors* (*HSFs*) are the key transcriptional regulators of heat response, capable of activating a large set of heat shock proteins (HSP) which act as chaperones in protein folding to preserve protein stability and functionality during stress (Kotak et al. 2007). The *HSF*s are key players in the heat response and the overexpression of a wheat HSF (*TaHsfA6b*) confers enhanced thermotolerance in barley through the upregulation of HSPs, LEA protein genes, and genes related to anti-oxidative enzymes (Kumar Poonia et al. 2020). Furthermore, it is remarkable that in barley allelic diversity in a specific heat shock protein gene (*HSP17.8*) has been found associated to the number of grains per spike and thousand grain weight (Xia et al. 2013).

In a doubled-haploid population obtained from the cross of a stay-green genotype and a malting variety, six and four QTLs for heat and drought stress, respectively, have been found, strengthening the idea of a link between the stay-green phenotype and tolerance to heat and drought (Gous et al. 2016). Besides, a QTL for grain yield under heat stress has been mapped on chromosome 2H in a genome-wide association study in a collection of spring barleys (Ingvordsen et al. 2015).

4.5 Genetics and Physiology of Salt Tolerance

High soil salinity is a major environmental limitation to the productivity of crop plants, especially in arid and semi-arid regions. More than 6% of the world's total land area is affected by soil salinity, and climate change and irrigation are expected to negatively influence this number in the future (Dagar et al. 2016). Under high salt conditions, available water is reduced because of the increase in the osmotic potential of soil; in addition, physiological activities (e.g., transpiration, photosynthesis) are impaired by the toxic effect of higher concentration of some ions or ion stress imbalance (Tavakkoli et al. 2011; Negrao et al. 2017), which may result in a heavy reduction in biomass and productivity. On the other hand, plants have evolved different mechanisms of tolerance to salt stress: osmotic tolerance, ion exclusion and tissue tolerance (Roy et al. 2014; Ismail and Horie 2017 for a recent review of involved genes and molecular networks).

With its ability to withstand up to 250 mM NaCl, barley is the most salt tolerant cereal crop. Still diversity exists between different barley accessions for tolerance to high salt concentration and this diversity has been exploited to identify physiological and molecular components of this important trait. For example, it has been observed that already at sodium concentrations lower than 200 mM NaCl, tolerant barley genotypes can control xylem Na^+ loading, thus adjusting the osmotic potential and avoiding the accumulation of Na^+ in the leaf tissue, where photosynthesis may be disturbed (Zhu et al. 2017). Lower sodium accumulation in the flag leaf of tolerant genotypes with respect to sensitive ones was also observed by Hazzouri et al. (2018), despite no differences in Na^+ root uptake under salt stress. This suggests differences in the rate of sodium transport from roots to shoots and in sodium sequestration from xylem. Wu et al. (2015a, b) showed a positive correlation between barley salt tolerance and chlorophyll content in excised leaves and observed a more efficient vacuolar Na^+ sequestration in leaf mesophyll in tolerant genotypes; on the contrary, sensitive barleys accumulated higher levels of sodium in chloroplasts. No change in chlorophyll content under salt stress was also detected in a barley mutant, together with a lower oxidative stress, lower Na^+ concentration and the maintenance of steady-state levels of K^+ in roots with respect to the wild type (Kiani et al. 2017). Detoxification from stress-induced reactive oxygen species (ROS) plays in fact a significant role in preventing damages to plasma membrane transporters and preserving cytosolic K^+ homeostasis (Adem et al. 2014).

Salt stress tolerance is a polygenic trait that allows plants to grow and photosynthesize under saline conditions. The wide phenotypic variability observed for this trait in barley fostered genetic studies aiming at identifying the underlying QTLs and genes, through linkage and association mapping in both cultivated and wild accessions (Zhu et al. 2015; Xue et al. 2017, 2019; Shen et al. 2018; Hazzouri et al. 2018; Mwando et al. 2020; Saade et al. 2020). Genome-wide association studies (GWAS) for salinity tolerance in a USDA barley core-collection grown under saline conditions identified a major locus on chromosome 4H controlling Na^+ content in the flag leaf (Hazzouri et al. 2018). Tissue specific expression of the *High-Affinity K^+ Transporter HvHKT1;5* candidate gene for this locus was observed, that might be related to the ability of retaining K^+ and excluding Na^+ from leaves of salt tolerant genotypes. A second *HKT* gene was characterized in barley (*HvHKT2;1*), that confers higher growth rate under saline conditions when overexpressed in transgenic lines, with respect to wild type plants (Mian et al. 2011). Interestingly, two *HKT* genes were also indicated as candidate genes for the sodium exclusion loci *Nax1* and *Nax2* in *Triticum monococcum* (Munns et al. 2012).

Barley *HVP10*, coding for a vacuolar H^+-inorganic pyrophosphatase (V-PPase) has been suggested as candidate gene for the *HvNax3* QTL identified in the biparental population CPI-71284-48 (*H. vulgare* spp. *spontaneum*) x Barque-73 on chromosome 7H (Shavrukov et al. 2010, 2013). An increase of expression levels of

HVP10 was previously detected in barley root in response to NaCl (Fukuda et al. 2004), similar to the *V-PPaseHVP1*, the vacuolar H^+-*ATPaseHvHVA*-A and the Na^+/H^+ *antiporterHvNHX1*. Taken together these results point to an important role of these genes in sequestering toxic Na^+ ions in the vacuoles. Transgenic barley plants that overexpress a vacuolar pyrophosphatase from Arabidopsis (*AVP1*) showed an increased biomass and grain yield with respect to wild-type plants when grown in the field under salt stress conditions (Schilling et al. 2014). The barley sodium exclusion *HvNax4* locus was mapped on chromosome 1H in the Clipper × Sahara (Algerian barley landrace) mapping population, where co-segregates with *HvCBL4*, a barley homologue of the *SOS3* salinity tolerance gene of Arabidopsis that mediates cellular signalling under salt stress. The effect of *HvCBL4* o Na^+ concentration in barley shoot was highly dependent on the environmental conditions and there was no detectable induction of gene expression by salt treatment in both parents (Rivandi et al. 2011), hence decisive proof that *HvCBL4* is the *HvNax4* gene is currently lacking.

In addition to the above *Nax* loci, several QTLs for traits associated to salinity tolerance have been identified in different populations screened at different growth stages. Xue et al. (2017) examined the response of barley seedlings from the Nure × Tremois doubled-haploid population to different salinity conditions and detected a new major QTL for root length under salt stress on chromosome 7HS. When the same protocol was applied to screen a collection of European winter cultivars for salt and osmotic stress resistance, common loci were detected on chromosomes 1H, 5H and 6H (Xue et al. 2019). A panel of two-row spring barley cultivars was recently evaluated under salinity stress during the vegetative and the reproductive stages, under controlled and field conditions, respectively (Saade et al. 2020). No overlapping QTL were detected between the two growing conditions, suggesting a possible involvement of different tolerance mechanisms. Mano and Takeda (1997) already observed that the effect of salt stress and the involved resistance genes might change depending to the growth stage: germination and seedling stages are the most sensitive ones, however tolerance at the reproductive phase under field conditions is probably the most valuable for breeders. For all these newly detected loci, candidate genes have been proposed based on the physical position on the high-quality reference sequence of the cultivar Morex (Monat et al. 2019), and more useful information will be gathered by exploiting the broader genic and allelic variation from the recently developed barley pan-genome (Jayakodi et al. 2020).

As highlighted with the identification of *HvNax3* and *HvNax4*, barley landraces and wild relatives are important sources of salinity tolerance genes and alleles (Shen et al. 2018). For example, barley lines carrying an introgression from *H. vulgare* spp. *spontaneum* on chromosome 2H showed a 30% yield increase under saline conditions in field trials (Saade et al. 2016). Useful tolerance mechanisms can also be dissected in the secondary and tertiary gene pools of barley, as it has been recently shown in the wild halophyte sea barley (*Hordeum marinum*) (Huang et al. 2018; Saoudi et al. 2019). Despite improvements are needed for image-based, non-destructive methods that continuously evaluate growth rate under salt stress

(Saade et al. 2020), it is expected that high-throughput phenotyping methods will facilitate the study of the physiological, genetic and molecular bases of barley tolerance to salinity. More attention should then be paid on integrating and combining different mechanisms, pyramiding the involved genes and manipulating master regulators in order to develop advanced breeding materials more adapted to saline soils.

4.6 Advanced Breeding for Stress Tolerance

4.6.1 Breeding for Abiotic Stress Tolerance: Basic Concepts

Breeding for abiotic stress tolerance means targeting genetics to increase crops' ability to overcome a plethora of environmental factors that dictate suboptimal growing conditions without penalizing yield as there are profound connections between this latter trait and abiotic stress tolerance (Tardieu and Tuberosa 2010). Historically, yield has been the main target trait to improve in crops but breeding for abiotic stress tolerance has the potential to unlock the full yield potential in stressful environments. Consequently, breeding for yield and for abiotic stress tolerance are two sides of the same coin as these traits are deeply correlated in many crops. Depending on the target environments, breeding programs aimed at developing high-yielding varieties might focus on abiotic stress tolerance.

Usually, a breeding program aimed at improving abiotic stress tolerances, either using grain yield or other traits as main selection criteria, is more efficient if the development of new varieties is focussed on specific target zones with similar characteristics or mega-environments (Braun et al. 1996), which are typically defined examining environmental factors that dictate crop stresses such as water availability, ambient temperatures, latitude, cropping system etc. Consequently, breeding for abiotic stress tolerance implies to define and organise field trials in environments where factors that impose abiotic stresses are pervasive and, hopefully, constant over different seasons. Specific statistical tools that exploit the availability of existing field trial records might be used for clustering target zones and splitting breeding for different mega-environments (Laffont et al. 2013; Tinker et al. 2015; Neisse et al. 2018).

In cereals and in other crops, either traits conferring abiotic stress tolerance or grain yield are generally quantitative or complex. In a population these traits show continuous phenotypic variability and are controlled by a complex genetic architecture involving many genes interacting each other and with the environment (Falconer and Mackay 1996). The theory of quantitative genetics assumes that the variation of phenotypic values observed in a population depends on genetic differences among individuals (G), environmental factors (E) and on the interaction between genotypes and environment, (GxE). For a quantitative trait the total phenotypic variance observed in a population equals to the sum of the variance of

genetic effects ($\boldsymbol{\sigma_G^2}$), the variance of environmental effects ($\boldsymbol{\sigma_E^2}$) and the variance associated with the GxE interaction effects ($\boldsymbol{\sigma_{GE}^2}$).

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GE}^2 \tag{1}$$

Similarly, the genetic component of variance ($\boldsymbol{\sigma_G^2}$) may be further partitioned into three components, which are the variance of additive effects ($\boldsymbol{\sigma_A^2}$), the variance of dominance effects ($\boldsymbol{\sigma_D^2}$) and the variance of epistatic or interaction effects ($\boldsymbol{\sigma_I^2}$) and consequently, Eq. 1 becomes:

$$\sigma_P^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2 + \sigma_E^2 + \sigma_{GE}^2 \tag{2}$$

Broad sense heritability (H^2) measures the selection accuracy when plants having the highest genetic values are chosen by selecting directly for phenotypic values and is defined as:

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2} \tag{3}$$

As the additive genetic effects are the only fraction of genetic variance that can be transmitted to the offspring, it is often convenient to compute narrow sense heritability (h^2), which is equal to:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} \tag{4}$$

H^2 and h^2 describe the amount of phenotypic variation of a complex trait that can be attributed to genetic and additive effects, respectively. For a given trait, each population has its own $\boldsymbol{H^2}$ and $\boldsymbol{h^2}$, noteworthy broad and narrow sense heritability of a trait may differ among different populations.

Breeding values (BVs) are defined as the sum of the additive genetic effects of genes in an individual, and consequently their variance in a population is equal to σ_A^2. Overall, BVs describe the effects of genes that a plant can pass on to its progeny and can be used to rank individuals to mate for generating next progeny (Falconer and Mackay 1996).

4.6.2 Direct and Indirect Selection

The success of plant breeding programs relies on the effective selection of individuals carrying favourable alleles, which has been historically addressed using phenotypic selection that is the selection of individuals exhibiting the greatest

phenotypic values as parents to mate for generating next progeny. During the last 60 years, phenotypic selection has allowed to obtain great advancement in crop breeding, but on the other side the application of this approach to improve abiotic stress tolerance has many pitfalls. Traits conferring abiotic stress tolerance are difficult to measure in field trials and there are often low or null correlations between their phenotypic values measured in controlled and in field conditions. To overcome these constrains, breeding for abiotic tolerance in crops has been conducted in stressful environments using yield as main selection criterion. This approach has been largely criticized, as in general yield exhibits low heritability values in either normal or harsh environments and consequently phenotypic selection becomes ineffective and does not result in high rates of genetic gain, that is the amount of increase in crop or trait performance that is achieved annually through artificial selection (Falconer and Mackay 1996; Jackson et al. 1996). Moreover, there are often negative correlations between yield and abiotic stress tolerance that impose to set trade-offs during selection.

Direct selection is an alternative way to improve abiotic stress tolerance in crops and allows overcoming some of the aforementioned pitfalls. The foundation of direct selection is to consider exclusively the phenotypic values of the target trait that must be improved as principal criterion. Using this approach superior genotypes exhibiting higher phenotypic mean are selected and mated to generate the next progeny. The selection response R, that is the between-generation change of the phenotypic mean due to selection, is quantified using the well-known breeders' equation:

$$R = h^2 S = \frac{\sigma_A^2}{\sigma_P^2} S \tag{5}$$

where h^2 is the narrow sense heritability of the trait (that is the covariance between the observed phenotypic values and the unknown breeding values) and S is the selection differential (the mean phenotypic value μ_s of the individuals selected expressed as a deviation from the population mean μ_P or parental generation), while $\sigma_A^2 a$ and σ_P^2 are the additive and phenotypic variances of the trait, respectively (Falconer and Mackay 1996). Equation 5 can be re-rewritten as follows:

$$R = h^2 S = ih\sigma_A \tag{6}$$

where $i = \frac{S}{\sigma_P}$ points out the selection intensity.

In the context of a breeding program, direct selection represents a straightforward way to improve traits exhibiting high heritability and that can evolve in response to selection. Differently, if the value of h^2 is low, the confounding environmental effects are high, and it is difficult to achieve a strong response to selection. Unfortunately, many agronomically important traits exhibit low levels of

heritability, making direct phenotypic selection ineffective for developing superior genotypes.

Indirect selection is exerted on a target trait indirectly, by means of selection applied to a second trait. The rationale is the correlation between two traits, which can arise owing to genetic or environmental factors (Falconer and Mackay 1996). Let us consider the phenotypic correlation r_p between a target trait and a secondary trait. r_p can arise as the breeding values of target and secondary traits are correlated, that is there is a genetic component that allows both traits to be negatively or positively correlated. Similarly, the correlation between two traits can arise owing to environmental factors (e.g. in cereal crops, the phenotypic correlation between plant height and grain yield can arise using different nitrogen levels in different trials). Consequently, the phenotypic correlation r_p depends on r_a, that is the correlation of the breeding values of the two traits, and r_e, that is the correlation of the environmental values, according to the following equation:

$$r_P = r_A h_X h_Y + r_E\left(1 - h_X^2\right)\left(1 - h_Y^2\right) \tag{7}$$

where h_X^2 and h_Y^2 point out the narrow sense heritability of trait x (e.g., the target trait) and y (e.g., the secondary trait), respectively (Falconer and Mackay 1996). Equation 7 shows that if the heritability values are high for both traits, then the correlation in breeding values controls the observed phenotypic correlation between trait x and trait y. Selection of a trait can cause a within generation change in the mean of a phenotypically correlated trait but for observing a between-generation change of the phenotypic mean, the breeding values of both traits must be correlated. This phenomenon is called correlated response to selection and underlies indirect selection. In other words, the rationale of indirect selection is the correlation between the breeding values of two traits, which often arises because genes that control one trait can have effects on other traits, a phenomenon known as pleiotropy, or owing to genetic linkage between genes controlling these traits. Considering two traits, namely trait x and trait y, the correlated response to selection on trait y ($\boldsymbol{CR_y}$) can be quantified as follows:

$$CR_y = ih_x h_y r_A \sigma_{Py} \tag{8}$$

where $\boldsymbol{r_A}$ is the correlation between the breeding values of trait x and trait y, while $\boldsymbol{i}$ is the intensity of selection applied to trait x and $\boldsymbol{\sigma_{Py}}$ points out the standard phenotypic deviation of trait y.

Indirect selection is effective when the target trait, that is the trait selected indirectly, exhibits low heritability while the correlated traits show higher values of heritability and is also convenient when the phenotypic values of the target trait are difficult to measure. In this latter case, indirect selection based on genetically correlated traits of easy measurement, is a rather interesting alternative to maximise the accuracy of phenotypic selection. An example of indirect breeding is to select for grain yield using morphological and physiological traits. Following this

approach, high yielding genotypes of durum wheat have been achieved in part by selecting for grain yield and by selecting for morphological traits positively correlated with grain yield (Peng et al. 2008; Lynch 2013). In other crops, seminal root system (Fig. 4.3) and other below-ground traits have been used for indirectly selecting high-yielding and drought tolerant genotypes for dry environments (Manschadi et al. 2008). While for grain yield, there are plenty of traits that can used for indirect selection, in barley limited knowledge has been acquired on traits that might be exploited for indirect selection of genotypes tolerant to abiotic stresses. Recently, a set of 13 physiological parameters have been pointed out as promising traits to identify drought-tolerant genotypes in barley, particularly leaf sap osmolality and relative water content at the seedling stage (Cai et al. 2020). Anyway, the implementation of these results for indirect selection of drought-tolerant genotypes in actual breeding programs can be hampered, as these traits cannot be currently scored as easily as required.

Fig. 4.3 Variability of the seminal root system in twenty-four barley genotypes. Seminal root number along with other below-ground traits are native traits of cereal crops that have been targeted to increase tolerance to drought stress and grain yield using indirect selection

4.6.3 Trade-Offs to Select for Multiple Traits

Breeding programs are not limited to the improvement of a single trait but might target many traits simultaneously. In most cases, breeding for abiotic stress tolerance might imply to determine trade-offs between traits conferring stress tolerance and yield as these traits are often negatively correlated in stressful environments (Tardieu and Tuberosa 2010). For instance, tolerance mechanisms against drought are most often physiologically associated with a reduction of biomass accumulation, hence yield potential (Blum 2009). Considering two traits and Eq. 8, the response to selection (***CR***) of trait x depends also on the correlation between the breeding values of trait x and y as follows:

$$CR_y = i_x h_x h_y r_A \sigma_{Py} \tag{9}$$

$$CR_x = i_y h_x h_y r_A \sigma_{Px} \tag{10}$$

These equations show that the correlated selection response $\boldsymbol{CR_y}$ on trait y depends on the selection intensity $\boldsymbol{i_x}$ applied to trait x and of the correlation of breeding values between trait x and y (Falconer and Mackay 1996).

If $\boldsymbol{r_A} = 0$ traits x and y are not genetically correlated and both traits are independent: this means that a selection differential applied to the first trait x does not affect the mean phenotypic value of the second trait y in the progeny, unless a selection differential is applied also to the second trait. If $\boldsymbol{r_A} > 0$ or $\boldsymbol{r_A} < 0$ traits x and y are positively or negatively correlated, respectively. In these conditions a selection differential applied on trait x causes a response to selection also for trait y, regardless whether a selection differential is deliberately applied to trait y or not. If traits are positively correlated, selection increases the mean phenotypic values of both traits in the next progeny, otherwise selection changes the mean phenotypic values of traits in opposite directions. In barley (and wheat), this latter case is generally observed between grain yield and abiotic stress tolerance and implies trade-offs during selection. Consequently, selection for multiple traits requires to establish different weights and priorities for each target trait and this issue has been often addressed using index selection.

Considering multiple traits, the net genetic merit M of one individual is defined as the linear combination of the breeding values of the considered traits weighted by their respective economic values as follows:

$$M = w^T g \tag{11}$$

where w^T is the transposed vector of trait economic values and g is the vector of trait breeding values (Céron-Rojas and Crossa 2018). In practice, the vector g is

unknown and is replaced by the vector of trait phenotypic values y to form the linear phenotypic selection index I as follows:

$$I = b^T y \tag{12}$$

where b^T points out the transposed vector of coefficients. Notice that both I and M are univariate random variables and that for maximising the correlation between M and I the vector b must be chosen appropriately. The advantage of I for making selection is that it allows selecting for multiple traits using the theory developed for univariate trait selection, that is the response of selection $\boldsymbol{R_I}$ is equal to:

$$R_I = h^2 S = ih\sigma_H = ir_{HI}\sigma_H^2 \tag{13}$$

where σ_H^2 is the variance of the net genetic merit in the population, r_{HI} is the Pearson's correlation between I and M and i points out the intensity of selection, that is $i = \frac{S}{\sigma_H}$. Equation 13 points out that for maximising the response of selection on I, $\boldsymbol{r_{HI}}$ must be maximized, that is the vector b must maximize the correlation between the net genetic value and the linear phenotypic selection index. In barley, several indices have been proposed to help selecting for drought stress tolerance and grain yield: e.g., stress susceptibility index (Fischer and Maurer 1978), water stress index (Rizza et al. 2004). Although the theory of selection index traces back to the last century, it has recently rediscovered and used in combination with genomic selection in cereal crops as explained later (Céron-Rojas and Crossa 2019, 2020; Moeinizade et al. 2020).

4.6.4 Genomic Selection to Improve Abiotic Stress Tolerance

The use of molecular markers is revolutionising crop breeding and, in recent years, a great number of loci for quantitative traits that underlie abiotic stress tolerance have been described. Given the quantitative nature of abiotic stress tolerance, the selection for specific loci or QTLs through classical marker assisted selection has only a limited success (Shamsudin et al. 2016), while Genomic selection (GS) represents the most promising approach. GS aims to predict the so called genomic estimated breeding values (GEBVs) of individuals (Desta and Ortiz 2014). Likewise the estimated BVs used in the animal model, GEBVs point out the genetic merit of individuals that might be mated for the following generation but are computed using molecular markers. In GS, the prediction of GEBVs is carried out using a training population (TP) of plants and appropriate statistical models for fitting the observed phenotypic values to the genomic profiles of TP individuals. Models developed using TPs are subsequently used to predict GEBVs for individuals that have only genotypic information.

Currently, two main types of statistical models have been deployed for GS. The first one encompasses whole-genome regression models, which aim to regress markers on phenotypic observations for estimating marker effects and computing GEBVs. The second type of models used in GS is based on linear mixed effects model (LMM). In LMM, markers are treated as random variables and their effects are predicted to compute GEBVs. Alternatively, instead of considering markers as random variables, LMM can be used for treating the unknown GEBVs of plants as random effects, which are estimated using a genomic relationship matrix (van Raden 2008). This latter approach is computationally more efficient than whole-genome regression methods and is named "Genomic Best Linear Unbiased Prediction" (GBLUP) method (van Raden 2008) and a simplified mathematical introduction of GS using GBLUP statistical model is here presented. Using matrix notation, the vector of phenotypic observations $\boldsymbol{y}$ for a given trait can be written using the following mixed linear model:

$$y = X\beta + Zu + e \tag{14}$$

where X is the incidence matrix of fixed effects, β is the vector of fixed effects, Z is the incidence matrix of random effects, u is the vector of random effects and e is the vector of residual error terms. β points out any fixed effect that might affect the phenotypic observations (e.g., location or year), while u points out the breeding values of individuals, which are treated as random effects. Equation 14 assumes $u \sim N(0, G)$, that is the vector of random genetic effects follows a multivariate normal distribution with mean 0 and variance-covariance structure equals to G matrix. Similarly, it assumes that $e \sim (0, R)$, where $R = I\sigma_e^2$, that is the error are independently normally distributed with mean 0 and variance equals to σ_e^2. Moreover, it supposes that $\text{cov}(u, e) = 0$, that is that the vectors of random effects and of model errors are independent and not correlated. Generally, y, X and Z are known, while β and u must be estimated and predicted, respectively. Henderson's mixed model equations (Henderson 1984) provide the mathematical solutions to compute the estimators of β and u as follows:

$$\begin{bmatrix} X^T R^{-1} X & X^T R^{-1} Z \\ Z^T R^{-1} X & Z^T R^{-1} Z + G^{-1} \end{bmatrix} \begin{bmatrix} \hat{\beta} \\ \breve{u} \end{bmatrix} = \begin{bmatrix} X^T R^{-1} y \\ Z^T R^{-1} X \end{bmatrix} \tag{15}$$

which leads to:

$$\hat{\beta} = \left(X^T V^{-1} X\right)^{-1} X^T V^{-1} y \tag{16}$$

$$\hat{u} = GZ^T V^{-1} \left(y - X\hat{\beta}\right) \tag{17}$$

where $V = ZGZ^T + I\sigma_e^2$. In the standard animal model (Henderson 1984), the covariance matrix G of additive effects is modelled as $G = \sigma_A^2 A$, where σ_A^2 is the variance of additive effects and A is the additive genetic relationship matrix, which

is usually based on pedigree, that is using expected relatedness between individuals. This *A* matrix does not consider the actual genetic relationships between individuals as mendelian sampling causes deviation from the expected relatedness. In GS, the matrix *A* is replaced with the matrix of genomic relationship *K,* which is constructed using molecular markers. Differently from matrix *A*, *K* measures the relatedness between individuals considering mendelian sampling and allows to better estimate $\hat{u}$ according to Eq. 17 (van Raden 2008). The approach described above to predict the genetic merit of individuals is named genomic BLUP (GBLUP) and is one of the most popular method used for implementing GS in plants and animals (Desta and Ortiz 2014). GBLUP method predicts GEBVs, which are used to predict the genetic merit of individuals. GEBVs provide a marker-based estimation of the likelihood of each individual to have a superior phenotype, eliminating the need to identify and map individual marker effects having statistical significance. Setting GS requires a TP for which both phenotypic and genotypic data are available to build a prediction model, which is subsequently applied to compute GEBVs of individuals that only have genotypic data.

Accuracy of GS is a key parameter to estimate its usefulness in plant breeding and provides measurable criteria for comparing different GS models. Prediction accuracy (r_A) of GS models is usually measured using the Pearson's correlation (r) between the GEBVs and the true breeding values according to the following equation:

$$r_A = \sqrt{\frac{h^2}{h^2 + \frac{M_e}{N_p}}} \tag{18}$$

True breeding values are unknown values and, to compute accuracy, they are usually replaced by appropriate phenotypic measurements (e.g., the adjusted means of traits). Consequently, the accuracy of GS is the correlation between GEBVs and observed phenotypes in genotypes that were not used for building the prediction model. Depending on the number of individuals used for building the GS model, there are several approaches to estimate model accuracy. The first approach uses validation populations (VPs), which refer to sets of individuals not included in the TP and for which genotypic and phenotypic information are available. In this case, the GS model constructed with the TP is used to predict the GEBVs of the VP, which are subsequently correlated with the phenotypic values. This approach requires to genotype and phenotype an additional set of individuals, alternatively, cross-validation schemes have been developed to estimate model accuracy without investing resources for characterizing extra individuals (Gianola and Schon 2016).

There is mounting evidence showing that for complex traits GS outperforms phenotypic selection and pure marker assisted selection when applied to different crops (De Los Campos et al. 2009, Jannink et al. 2010). Furthermore, selection based on GEBVs has the advantage to shorten breeding cycle duration as it is no longer necessary to wait for late filial generations to phenotype for quantitative traits such as yield and or its component traits. GS makes more efficient

early-generation selection for yield and other traits and allows to improve breeding efficiency by reducing the number of genotypes to be tested in subsequent, expensive trials, thereby increasing genetic gain per unit cost.

It is worth mentioning that several theorical and empirical experiments have pointed out that GS imposes a strong response to selection (Hayes et al. 2009, Lorenzana and Bernardo 2009, Jannink et al. v). This can be easily explained following Eq. 6, considering that h points out the accuracy of phenotypic selection, that is the Pearson's correlation between an individual's breeding and phenotypic values. Replacing h with r_a it allows showing that, in the same conditions, the response to selection using GS is higher as its accuracy r_a is higher.

The use of GS for selecting traits that are known to confer abiotic stress tolerance is gaining momentum as it offers several advantages. As stated earlier, phenotyping for these traits that are known to improve abiotic stress tolerance is demanding and challenging in many crops. Nevertheless, phenotyping a TP of plants for developing GS might be affordable and pay down this investment once the GS model is applied to larger populations. While in barley this type of GS applications is lagging behind, in other cereal crops there are several examples that corroborate that this approach might be successful. In durum wheat, the architecture of below ground traits is correlated to the plant's ability to withstand drought stress under different climatic conditions (El Hassouni et al. 2018). Following this research line, in bread wheat GS has been applied to predict two root traits (total root length between 1.2 and 2 m depth and root length four intervals) using semi-field phenotyping facilities (Guo et al. 2020). Interestingly, these authors reported that GS models for predicting root traits with acceptable predictive ability can be deployed using only 84 winter wheat genotypes and demonstrated that GS might play a fundamental role for breeding for below-ground traits (Guo et al. 2020) or other traits indirectly correlated to abiotic tolerance.

4.6.5 Genomic Selection for Multiple Traits

This chapter has previously shown that owing to the genetic correlation between abiotic stress tolerance and other agronomically important traits (e.g. grain yield), breeding programs usually conduct multivariate trait selection, that is phenotypic selection on multiple traits. There are two main research lines that allow multivariate trait selection, and consequently breeding for abiotic stress tolerance: multi-trait genomic selection (MT-GS) and linear or constrained selection indices (Jia and Jannink 2012a).

Multi-trait genomic selection (MT-GS) models include genetically correlated traits and have been developed as the prediction accuracy of a target trait exhibiting low heritability can be improved if other traits with higher heritability are included for the prediction (Jia and Jannink 2012b). Expanding the univariate genomic selection model of GBLUP (Eq. 15) for two traits A and B, allows writing the following model:

$$\begin{bmatrix} y_A \\ y_B \end{bmatrix} = \begin{bmatrix} X_A & 0 \\ 0 & X_B \end{bmatrix} \begin{bmatrix} \beta_A \\ \beta_B \end{bmatrix} + \begin{bmatrix} Z_A & 0 \\ 0 & Z_B \end{bmatrix} \begin{bmatrix} u_A \\ u_B \end{bmatrix} + \begin{bmatrix} e_A \\ e_B \end{bmatrix} \tag{19}$$

where y_A and y_B are the vectors of phenotypes, X_A and X_B points out the incidence matrix of fixed effects, β_A and β_B are the vectors of fixed effects, Z_A and Z_B are the incidence matrices of random effects, u_A and u_B are the vector of random effects and e_A and e_b are the vectors of residual error terms for traits A and B, respectively. If the incidence matrices of fixed and random effects are the same for trait A and B, Eq. 20 becomes:

$$\begin{bmatrix} y_A \\ y_B \end{bmatrix} = \begin{bmatrix} X & 0 \\ 0 & X \end{bmatrix} \begin{bmatrix} \beta_A \\ \beta_B \end{bmatrix} + \begin{bmatrix} Z & 0 \\ 0 & Z \end{bmatrix} \begin{bmatrix} u_A \\ u_B \end{bmatrix} + \begin{bmatrix} e_A \\ e_B \end{bmatrix} \tag{20}$$

The residuals $\begin{bmatrix} e_A \\ e_B \end{bmatrix}$ are assumed to follow a normal distribution with $\begin{bmatrix} e_A \\ e_B \end{bmatrix} \sim N(0, I \otimes R)$ where $R = \begin{bmatrix} \sigma^2_{eA} & \sigma_{eAB} \\ \sigma_{eAB} & \sigma^2_{eB} \end{bmatrix}$ and that the vector $\begin{bmatrix} u_A \\ u_B \end{bmatrix} \sim N(0, G \otimes H)$ where $H = \begin{bmatrix} \sigma^2_{uA} & \sigma_{uAB} \\ \sigma_{uAB} & \sigma^2_{uB} \end{bmatrix}$ points out the variance-covariance matrix of the breeding values between the two traits.

Following GBLUP assumptions, Henderson's mixed model equations can be extended to multiple traits as follows:

$$\begin{bmatrix} X^T R^{-1} X & X^T R^{-1} Z \\ Z^T R^{-1} X & Z^T R^{-1} Z + G^{-1} \otimes G_0^{-1} \end{bmatrix} \begin{bmatrix} \hat{\beta} \\ \breve{u} \end{bmatrix} = \begin{bmatrix} X^T R^{-1} y \\ Z^T R^{-1} X \end{bmatrix} \tag{21}$$

where G_0 is the matrix of genetic covariance between trait A and B. The matrix $\boldsymbol{G}_0$ allows borrowing information from a correlated trait to better predict the main trait. Several studies have pointed out that multi-trait GS, either implemented with GBLUP or other statistical methods, outperforms univariate analyses in terms of prediction performance if the correlation between traits is moderate or large (Calus and Veerkamp 2011; Jia and Jannink 2012a). So far, in barley the application of multi-trait GS has been limited to improve malting quality and agronomically important traits (Bhatta et al. 2020). In this study a single trait and a multi trait GS models were fitted to a TP of 145 doubled haploid lines obtained from 5 different crosses, and subsequently the predictive ability of the resulting GS models was estimated using two different types of cross validation schemes. Comparison of single trait and multi trait GP models revealed that the latter one has higher values of predictive ability and that the use of correlated traits in GS has the potential to improve the prediction of quality traits. The results of this study have profound implications for breeding for abiotic stress resistance as curated phenotypic information on key traits along with genotyping data might allow predicting abiotic stress tolerance in large breeding populations without conducting expensive field trials.

The second approach that allows implementing GS for multiple traits is based on phenotypic selection index. The advantage of this approach is that the theory developed for univariate genomic selection can be applied treating indices as phenotypes and then allowing multiple-trait selection. So far, several indices have been constructed for abiotic stress tolerance. The application of GS using phenotypic indices have been used in wheat to conduct a simultaneous selection for major agronomic traits like grain yield and protein content which poses a major challenge in breeding due to frequently observed strong negative correlation between these traits (Michel et al. 2019a, b), a condition similar to the selection for traits conferring abiotic stress tolerance and grain yield.

4.7 Stress Tolerant Barley: The Upcoming Promise of Genome Editing

In plant science, the possibility to adopt site specific nucleases (SSNs) to specifically change selected genome sequences has opened the way to the development of novel breeding techniques. The common feature of these techniques is the specificity of the DNA cleavage and the formation of a Double Stranded Break (DSB); cells are then able to repair DSBs either through homology directed repair (HDR) or non-homologous end joining (NHEJ). While the HDR system opens the possibility for gene replacement, the NHEJ is an error-prone mechanism often resulting in knock-out mutations. The improvement of the genome editing (GE) technology has gone through the exploitation of different protein complexes that are able to cause DSBs in selected target sequences, nevertheless, today GE is mainly achieved through genetic scissors known as CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9). A tool developed studying the RNA mediated adaptive immune system in bacteria; through this system, bacteria are indeed able to cleave the pathogen's nucleic acid genome (Jinek et al. 2012). The type II CRISPR/Cas9 is a two-component system made of the Cas9 protein and the so-called single guide RNA (sgRNA). Here, the Cas9 protein owns the nuclease activity while the sequence specific DNA cleavage is obtained through the complementarity between 20 nucleotides positioned at the 5′ end of the sgRNA and the target DNA. An important requirement for the system to work, is the presence of a 5′-3′ protospacer-adjacent motif (PAM) contiguous to the DNA target sequence (Subburaj et al. 2016). When the Cas9 protein and the sgRNA are co-expressed in the same cells, the sgRNA is loaded into the Cas9 thus leading the cleavage of the complementary DNA target which is contiguous to the PAM sequence. Once the DSB is induced, gene replacement or different mutations (mainly small insertions or deletions, indels) are induced by the HDR or NHEJ systems, respectively.

Scientists can choose between Cas9 proteins with different origins and/or engineered to recognize diverse PAM sequences such as VQR-Cas9 (NGA PAM), EQR-Cas9 (NGAG PAM), VRERCas9 (NGCG PAM), SaKKH-Cas9 (NNNRRT

PAM) (Kleinstiver et al. 2015), xCas9 (NG, GAA, and GTA PAM) (Hu et al. 2018) 44) and SpCas9-NG (NG PAM) (Nishimasu et al. 2018). Recently, the manipulation of one or both the Cas9 catalytic domains led to the development of more sophisticated GE systems. In the base editing system, the inactivation of a single catalytic domain led to the formation of the nCas9 protein that works as a nickase (Komor et al. 2016). The nCas9 is then flanked by either a cytidine deaminase or an adenosine deaminase thus opening the possibility to specifically change C:G to T:A (C-base editor) or A:T to C:G (A-base editor) (Hess et al. 2017). In this case, a selected single nucleotide within the target sequence is specifically changed into another thus leading to potential modification of protein sequences. When the Cas9 catalytic domains are both inactivated the obtained dCas9 protein is not able to induce any DSB, but it works as a platform to recruit other transcriptional regulators or fluorescent proteins at specific target sites (Jiang and Doudna 2017). As an alternative to the Cas9 protein, a CRISPR/Cpf1 system is available (Wang et al. 2017). Differently from the Cas9 protein, Cpf1 uses a T-rich PAM sequence, it generates cohesive ends and it cuts distant and downstream from the PAM; in some cases, a more efficient HDR was observed (Tang et al. 2017).

Beside creating DSBs in selected target sequences, mutations at unintended targets (known as off-targets) can be observed with different frequency depending on the DSBs GE technology and on the conservation of the target sequence within the genome. Different bioinformatic platforms support the precise selection of the target site (https://crispr.i2bc.paris-saclay.fr/Server/) as well as the off-target prediction (http://www.rgenome.net/cas-offinder/).

GE constructs are delivered into the plants obtaining a stable or transient expression of the CRISPR/Cas9 DNA. *Agrobacterium*-mediated transformation and particle bombardment lead to stable transformation while protoplast transfection is used for transient expression. In case of stable transformation, DNA segregation allows to identify transgene free plants already in the T_1 generation; in these plants, the construct carrying the Cas9 protein, the sgRNA sequences and the selectable marker is lost by segregation, but the desired mutation is maintained. Recently, a DNA-free GE system has been developed in wheat through the delivery of Cas9 ribonucleoproteins and the sgRNA (Zhang et al. 2016). This strategy avoids the issue of DNA inserted within the host genome and saves the time needed for backcrossing.

GE is revolutionizing plant science and is making possible to create targeted biodiversity. Indeed, GE represents an efficient method for pyramid breeding opening the possibility of combining different beneficial alleles in one single plant. This opportunity is extremely appealing when considering the risk of negative alleles introgressed from wild progenitors or landraces along with novel useful alleles due to linkage disequilibrium. Stable transformation for over-expressing useful alleles represents an option that has been explored to confer stress tolerance, but the choice of the most suitable promoter is challenging, since strong promoters, although enhance stress tolerance, might be detrimental for plant development and the full expression of yield potential. In this perspective, GE allows to create a set of allelic variants in the promoter regions and to select those causing the most

convenient expression of the regulated genes. Since the process of domestication led to an important reduction in biodiversity, GE might be used to re-create wild alleles in domesticated genotypes as well as for a de novo domestication of wild varieties.

The possibility to exploit the GE technology depends on three essential conditions as the availability of (i) a reference genome, (ii) efficient transformation and regeneration protocols, preferably suitable for different genotypes and (iii) the identification and functional characterization of key genes controlling the desired traits. In barley, these features are fulfilled but improvements are needed to expand GE applications and pyramiding of alleles in a wide range of genotypes.

A good assembly of the barley reference genome is available since 2017 (Mascher et al. 2017) and more barley genomes have been recently released including the genome of transformation reference cultivar Golden Promise (Schreiber et al. 2020) and of the first barley pan-genome made of 20 accessions (landraces, cultivars and a wild barley) that are considered representatives of the global barley diversity (Jayakodi et al. 2020).

Stable and transient transformations are easily achieved in barley although a good transformation efficiency (around 25%) is possible only for the cultivar Golden Promise (Bartlett et al. 2008) using *Agrobacterium*-mediated transformation of immature embryos. The development of novel protocols to transform and regenerate diverse barley varieties is still a priority, this aim must be reached to throw open the doors to alleles pyramiding in already commercialized barley genotypes or for de novo domestication strategies. Some promising results have been recently published. Orman-Ligeza et al. (2020) have identified the *TRA1* genetic locus in the *lys3* mutant background which seems to positively influence the regeneration efficiency of barley transformed embryos. Introgression of this locus into selected genotypes might be the starting point to obtain a wider range of transformable barley accessions. An anther culture-based system has been recently developed that allowed to create transgenic and gene-edited plants from diverse Australian commercial barley varieties (Han et al. 2021). In this case, the double haploid resultant plants are expected to be homozygous for the transgene insertion; this requires a backcross to allow the segregation of the construct carrying the Cas9/sgRNA sequences and the selectable marker.

A good number of vectors designed for GE applications in cereals already exist (https://www.addgene.org/crispr/plant/). No specific barley promoters must be incorporated into these vectors since the maize and wheat ubiquitin promoters (*pZmUbi* and *pTaUbi*) as well as the rice and wheat U3 and U6 promoters (*pOsU3*, *pOsU6*, *pTaU3* and *pTaU6*) successfully worked upstream the Cas9 cDNA sequence and the sgRNAs respectively in the Golden Promise donor background (Lawrenson et al. 2015). Nevertheless, an alternative version of the RNA guided Cas9 system designed for genome editing in barley was recently proposed by Gasparis and colleagues (2018) that successfully worked for simplex and multiplex editing.

Successful GE relays on the identification and detailed functional characterization of genes with a key role in abiotic stress tolerance. In barley, many loci contributing to abiotic stress resistance have been identified through quantitative

genetic, buy only few causal genes were described. In the literature there are functional studies reporting the effects of the over-expression of selected genes in conferring an increased resistance to specific stress conditions (see the previous sections of this chapter for the description of *HvCBF*, *HvHKT1;5* and *HvHKT2;1*, *HvEPF1*, *CesA1* and *HVA1*) as well as examples of allelic variants contributing to stress resistance (see the previous sections of this chapter for the description allelic variants for *HvCBF14*, *HvCBF10A*, *HvCBF10B*, *P5cs1* and *HSP17.8*). Base editing, promoter variants or gene replacement can used to re-create useful alleles in elite cultivars. Based on this knowledge, a short list of candidate genes can be suggested as targets for GE. Table 4.1 reports examples of modification at selected genes/regulatory regions that could be exploited with GE with the final aim of developing barley plants resistant to diverse abiotic stresses.

Table 4.1 Examples of modification at selected gene/regulatory regions that could be exploited with GE to develop barley plants resistant to diverse abiotic stresses

Candidate genes	GE approaches	Target abiotic stress
Selected *HvCBF* promoters	1. GE to increase expression 2. Inducible dCas9 linked to selected transcriptional activators	Cold
Promoter region of *HvHKT1;5* and *HvHKT2;1* (Mian et al. 2011) and *HvAVP1* (Schilling et al. 2014)	1. GE to increase expression 2. Inducible dCas9 linked to selected transcriptional activators	Salinity
HvDRO1 promoter	CRISPR/Cas9 to release the auxin negative control on *DRO1*	Drought
Promoter regions of *DHN8*, *DHN5*, (LEA genes), *DREB* (Morran et al. 2011) and *HVA1* gene (Sivamani et al. 2000)	1. GE to increase expression 2. Inducible dCas9 linked to selected transcriptional activators	Drought
HvCBF10B and *HvCBF10A*	CRISPR/Cas9 to perform HDR to create the alleles described in Reinert et al. (2016)	Drought
Promoter region of the *HvP5cs1* gene	CRISPR/Cas9 to perform HDR to create the alleles described in Muzammil et al. (2018)	Drought
HSP17.8	CRISPR/nCas9 to perform base editing to introduce the SNPS described in Xia et al. (2013)	Heat
Promoter of *Hv-miR827* (Ferdous et al. 2017)	1. GE to increase expression 2. Inducible dCas9 linked to selected transcriptional activators	Drought
Promoter of *HvEPF1* (Hughes et al. 2017)	1. GE to increase expression 2. Inducible dCas9 linked to selected transcriptional activators	Drought
HSP17.8 (Xia et al. 2013)	CRISPR/Cas9 to perform HDR to create the alleles described in (Xia et al. 2013)	Heat

4.8 Conclusions

The impact of climate change on agriculture, particularly in some regions, e.g., the Mediterranean basin where barley is a traditional crop, is posing new challenges for barley breeding and the selection of varieties resilient to abiotic stresses and capable of a more efficient use of available resources is urgent.

The huge amount of knowledge that have been acquired after many years of research described the genetic diversity available for stress tolerance in cultivated and wild barley and indicated many loci and genes involved in stress response and stress tolerance. Nevertheless, due to the multigenic nature of abiotic stress tolerance, the introduction of one or few genes or QTLs in elite germplasm, as it is usually done with standard marker assisted selection, may result in a subtle phenotypic effect or yield increase. Furthermore, with few exceptions, no single trait is enough to guarantee the yield under the different stress conditions that a plant experience in different years. Indeed, despite many works dedicated to identifying the genetic bases of barley resilience to abiotic stresses, there is a large gap between the knowledge accumulated and the genetic progress achieved by breeding.

During the last decade a large investment on new technologies such as genomic selection, high-throughput genotyping and genome editing, as well as a general advance in the understanding the barley genome with the publication of the pan-genome of barley (Jayakodi et al. 2020) is opening new opportunities for barley breeding. Although phenotyping still represents a limiting factor in the identification of the most tolerant genotypes, the selection of more stress tolerant cultivars is now more feasible than in past years.

References

Adem GD, Roy SJ, Zhou M, Bowman JP, Shabala S (2014) Evaluating contribution of ionic, osmotic and oxidative stress components towards salinity tolerance in barley. BMC Plant Biol 14:113

Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C3 cereals: what should we breed for? Ann Bot 89:925–940

Ashraf A, Rehman OU, Muzammil S, Léon J, Naz AA, Rasool F, Ali GM, Zafar Y, Khan MR (2019) Evolution of *Deeper Rooting 1*-like homoeologs in wheat entails the C-terminus mutations as well as gain and loss of auxin response elements. PLoS Onr 14:e0214145

Badawi M, Reddy YV, Agharbaoui Z, Tominaga Y, Danyluk J, Sarhan F, Houde M (2008) Structure and functional analysis of wheat *ICE* (inducer of *CBF* expression) genes. Plant Cell Physiol 49:1237–1249

Bartlett JG, Alves SC, Smedley M, Snape JW, Harwood WA (2008) High-throughput *Agrobacterium*-mediated barley transformation. Plant Methods 4:22

Bellard C, Bertelsmeler C, Leadley P, Thuiller W, Courchamp F (2012) Impacts of climate change on the future of biodiversity. Ecol Lett 15:365–377

Bhatta M, Gutierrez L, Cammarota M, Cardozo F, Germán S, Gómez-Guerrero B, Pardo MF, Lanaro V, Sayas M, Castro AJ (2020) Multi-trait genomic prediction model increased the

predictive ability for agronomic and malting quality traits in barley (*Hordeum vulgare* L.). G3 Gene Genomics Genet:1113–1124
Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Aust J Agric Res 56:1159–1168
Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crop Res 112:119–123
Bodner G, Nakhforoosh A, Kaul H-P (2015) Management of crop water under drought: a review. Agron Sustain Dev 35:401–442
Braun HJ, Rajaram S, Van Ginkel M (1996) CIMMYT's approach to breeding for wide adaptation. Euphytica 92:175–183
Bustos-Korts D, Dawson IK, Russell J, Tondelli A, Guerra D et al (2019) Exome sequences and multi-environment field trials elucidate the genetic basis of adaptation in barley. Plant J 99:1172–1191
Cai K, Chen X, Han Z, Wu X, Zhang S, Li Q, Nazir MM, Zhang G, Zeng F (2020) Screening of worldwide barley collection for drought tolerance: the assessment of various physiological measures as the selection criteria. Front Plant Sci 11:1159
Calus MPL, Veerkamp RF (2011) Accuracy of multi-trait genomic selection using different methods. Genet Sel Evol 43:1–14
Campoli C, Shtaya M, Davis SJ, von Korff M (2012) Expression conservation within the circadian clock of a monocot: natural variation at barley Ppd-H1 affects circadian expression of flowering time genes, but not clock orthologs. BMC Plant Biol 12:97
Cantalapiedra CP, García-Pereira MJ, Gracia MP, Igartua E, Casas AM, Contreras-Moreira B (2017) Large differences in gene expression responses to drought and heat stress between elite barley cultivar scarlett and a spanish landrace. Front Plant Sci 8:647
Casas AM, Djemel A, Ciudad FJ, Yahiaoui S, Ponce LJ et al (2011) *HvFT1* (*VrnH3*) drives latitudinal adaptation in Spanish barleys. Theor Appl Genet 122:1293–1304
Cattivelli L (2011) More cold tolerant plants in a warmer world. Plant Sci 180:1–2
Cattivelli L, Bartels D (1990) Molecular cloning and characterization of cold-regulated genes in barley. Plant Physiol 93:1504–1510
Cattivelli L, Crosatti C (2020) Conducting field trials for frost tolerance breeding in cereals. In: Zuther E, Hincha DK (eds) Methods in molecular biology: plant cold acclimation, 2nd edn. Humana Press, New York, USA, pp 43–52
Cattivelli L, Rizza F, Badeck F-W, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res 105:1–14
Céron-Rojas JJ, Crossa J (2018) Linear Selection Indices in Modern Plant Breeding. Springer-Nature.
Cerón-Rojas JJ, Crossa J (2019) Efficiency of a constrained linear genomic selection index to predict the net genetic merit in plants. G3 Genes Genomics Genet 9:3981–3994
Cerón-Rojas JJ, Crossa J (2020) Combined multistage linear genomic selection indices to predict the net genetic merit in plant breeding. G3 Genes Genomics Genet 10:2087–2101
Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. Funct Plant Biol 30:239
Choi D-W, Zhu B, Close TJ (1999) The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 *Dhn* genes of cv Dicktoo. Theor Appl Genet 98:1234–1247
Comadran J, Kilian B, Russell J, Ramsay L, Stein N et al (2012) Natural variation in a homolog of *Antirrhinum CENTRORADIALIS* contributed to spring growth habit and environmental adaptation in cultivated barley. Nat Genet 44:1388–1392
Comstock JP (2002) Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. J Exp Bot 53:195–200
Contreras-Moreira B, Serrano-Notivoli R, Mohammed NE, Cantalapiedra C, Begueria S, Casa A, Igartua E (2017) Genetic association with high-resolution climate data reveals selection

footprints in the genomes of barley landraces across the Iberian Peninsula. Mol Ecol 28:1994–2012

Crosatti C, Soncini C, Stanca AM, Cattivelli L (1995) The accumulation of a cold-regulated chloroplastic protein is light-dependent. Planta 196:458–463

Dagar JC, Sharma PC, Chaudhari SK, Jat HS, Ahamad S (2016) Climate change vis-à-vis saline agriculture: Impact and adaptation strategies. In: Dagar JC, Sharma PC, Sharma DK, Singh AK (eds) Innovative saline agriculture. Springer, New Delhi, India, pp 5–53

Dal Bosco C, Busconi M, Govoni C, Baldi P, Stanca AM, Crosatti C, Bassi R, Cattivelli L (2003) Cor gene expression in barley mutants affected in chloroplast development and photosynthetic electron transport. Plant Physiol 131:793–802

De Los Campos G, Naya H, Gianola D, Crossa J, Legarra A, Manfredi E, Weigel K, Cotesal M (2009) Predicting quantitative traits with regression models for dense molecular markers and pedigree. Genetics 182:375–385

Desta ZA, Ortiz R (2014) Genomic selection: Genome-wide prediction in plant improvement. Trends Plant Sci 19:592–601

Drosse B, Campoli C, Mulki A, von Korff M (2014) Genetic control of reproductive development. In: Kumlehn J, Stein N (eds) Biotechnological approaches to barley improvement. biotechnology in agriculture and forestry, vol 69. Springer-Verlag, Berlin, Heidelberg, pp 81–100

El Hassouni K, Alahmad S, Belkadi B, Filali-Maltouf A, Hickey LT, Bassi FM (2018) Root system architecture and its association with yield under different water regimes in durum wheat. Crop Sci 58:2331–2346

Ensminger I, Busch F, Huner NPA (2006) Photostasis and cold acclimation: sensing low temperature through photosynthesis. Physiol Planta 126:28–44

Falconer DS, Mackay TFC (1996) Introduction to Quantitative Genetics (Fourth Edition) Longman Group Ltd., London

Fan Y, Shabala S, Ma YL, Xu RG, Zhou MX (2015) Using QTL mapping to investigate the relationships between abiotic stress tolerance (drought and salinity) and agronomic and physiological traits. BMC Genom 16:43

Fang Z, Gonzales AM, Clegg MT, Smith KP, Muehlbauer GJ, Steffenson BJ, Morrell PL (2014) Genomic regions contribute disproportionately to geographic differentiation in wild barley. G3 Genes Genomics Genet 4:1193–1203

Ferdous J, Whitford R, Nguyen M, Brien C, Langridge P, Tricker PJ (2017) Drought-inducible expression of *Hv-miR827* enhances drought tolerance in transgenic barley. Funct Integr Genom 17:279–292

Feuillet C, Langridge P, Waugh R (2008) Cereal breeding takes a walk on the wild side. Trends Genet 24:24–32

Fischer RA, Maurer R (1978) Drought resistance in spring wheat cultivars. 1. Grain yield response. Aust J Agric Res 29:897–912

Fisk SP, Cuesta-Marcos A, Cistue L, Russell J, Smith KP, Baenziger S, Bedo Z, Corey A, Filichkin T, Karsai I Waugh R, Hayes PM (2013) FR-H3: a new QTL to assist in the development of fall-sown barley with superior low temperature tolerance. Theor Appl Genet 126:335–47

Francia E, Rizza F, Cattivelli L, Stanca AM, Galiba G, Toth B, Hayes PM, Skinner JS, Pecchioni N (2004) Two loci on chromosome 5H determine low-temperature tolerance in a 'Nure' (winter) x 'Tremois' (spring) barley map. Theor Appl Genet 108:670–680

Francia E, Barabaschi D, Tondelli A, Laidò G, Rizza F, Stanca AM, Busconi M, Fogher C, Stockinger EJ, Pecchioni N (2007) Fine mapping of a *HvCBF* gene cluster at the frost resistance locus *Fr-H2* in barley. Theor Appl Genet 115:1083–1098

Francia E, Morcia C, Pasquariello M, Mazzamurro V, Milc JA, Rizza F, Terzi V, Pecchioni N (2016) Copy number variation at the *HvCBF4–HvCBF2* genomic segment is a major component of frost resistance in barley. Plant Mol Biol 92:161–175

Fricano A, Rizza F, Faccioli P, Pagani D, Pavan P, Stella A, Rossini L, Piffanelli P, Cattivelli L (2009) Genetic variants of *HvCBF14* are statistically associated with frost tolerance in a European germplasm collection of *Hordeum vulgare*. Theor Appl Genet 119:1335–1348

Fukuda A, Chiba K, Maeda M, Nakamura A, Maeshima M, Tanaka Y (2004) Effect of salt and osmotic stresses on the expression of genes for the vacuolar H^+-pyrophosphatase, H^+-ATPase subunit A, and Na^+/H^+ antiporter from barley. J Exp Bot 55:585–594

Gasparis S, Kała M, Przyborowski M, Łyżnik LA, Orczyk W, Nadolska-Orczyk A (2018) A simple and efficient CRISPR/Cas9 platform for induction of single and multiple, heritable mutations in barley (Hordeum vulgare L.) Plant Methods 18:14–111

Gianola D, Schon CC (2016) Cross-validation without doing cross-validation in genome-enabled prediction. G3 Genes Genomics Genet 6:3107–3128

Gol L, Haraldsson EB, von Korff M (2021) *Ppd-H1* integrates drought stress signals to control spike development and flowering time in barley. J Exp Bot 72:122–136

Gous PW, Hickey L, Christopher JT, Franckowiak J, Fox GP (2016) Discovery of QTL for stay-green and heat-stress in barley (*Hordeumvulgare*) grown under simulated abiotic stress conditions. Euphytica 207:305–317

Guo X, Svane SF, Füchtbauer WS, et al (2020) Genomic prediction of yield and root development in wheat under changing water availability. Plant Methods 16:1–15

Haas M, Scheiber M, Mascher M (2018) Domestication and crop evolution of wheat and barley: genes, genomics, and future directions. J Integr Plant Biol 61:204–225

Han Y, Broughton S, Liu L, Zhang X-Q, Zeng J, He X, Li C (2021) Highly efficient and genotype-independent barley gene editing based on anther culture. Plant Comm 1:100082

Hanin M, Ebel C, Ngom M, Laplaze L, Masmoudi K (2016) New insights on plant salt tolerance mechanisms and their potential use for breeding. Front Plant Sci 7:1787

Hayes BJ, Bowman PJ, Chamberlain AJ, Goddard ME (2009) Invited review: Genomic selection in dairy cattle: Progress and challenges. J Dairy Sci 92:433–443

Hazzouri KM, Khraiwesh B, Amiri KMA, Pauli D, Blake T, Shahid M, Mullath SK, Nelson D, Mansour AL, Salehi-Ashtiani K, Purugganan M, Masmoudi K (2018) Mapping of *HKT1;5* gene in barley using GWAS approach and its implication in salt tolerance mechanism. Front Plant Sci 9:156

Hess GT, Tycko J, Yao D, Bassik MC (2017) Methods and applications of CRISPR-mediated base editing in eukaryotic genomes. Mol Cell 68:26–43

Honsdorf N, March TJ, Berger B, Tester M, Pillen K (2014) High-throughput phenotyping to detect drought tolerance QTL in wild barley introgression lines. PLoS One 9:e97047

Honsdorf N, March TJ, Pillen K (2017) QTL controlling grain filling under terminal drought stress in a set of wild barley introgression lines. PLoS One 12:e0185983

Hu H, Ahmed I, Choudhury S, Fan Y, Shabala S, Zhang G, Harrison M, Meinke H, Zhou M (2019) Wild barley shows a wider diversity in genes regulating heading date compared with cultivated barley. Euphytica 215:75

Hu JH, Miller SM, Geurts MH, Tang W, Chen L, Sun N, Zeina CM, Gao X, Rees HA, Lin Z, Liu DR (2018) Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature 556:57–63

Huang L, Kuang L, Li X, Wu L, Wu D, Zhang G (2018) Metabolomic and transcriptomic analyses reveal the reasons why *Hordeummarinum* has higher salt tolerance than *Hordeum vulgare*. Environ Exp Bot 156:48–61

Hughes J, Hepworth C, Dutton C, Dunn JA, Hunt L, Stephens J, Waugh R, Cameron DD, Gray JE (2017) Reducing stomatal density in barley improves drought tolerance without impacting on yield. Plant Physiol 174:776–787

Ingvordsen CH, Backes G, Lyngkjaer MF, Peltonen-Sainio P, Jahoor A, Mikkelsen TN, Jorgensen RB (2015) Genome-wide association study of production and stability traits in barley cultivated under future climate scenarios. Mol Breed 35:84

IPCC (2013) Summary for policymakers. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Doschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the

physical science basis. Contribution of working group I to the 5th assessment report of the intergovernmental panel on climate change, Cambridge University Press, Cambridge, UK
Ismail AM, Horie T (2017) Genomics, physiology, and molecular breeding approaches for improving salt tolerance. Annu Rev Plant Biol 68:405–434
Ivandic V, Hackett CA, Zhang ZJ, Staub JE, Nevo E, Thomas WTB, Forster BP (2000) Phenotypic responses of wild barley to experimentally imposed water stress. J Exp Bot 51:2021–2029
Jackson P, Robertson M, Cooper M, Hammer G (1996) The role of physiological understanding in plant breeding; From a breeding perspective. Field Crop Res 49:11–37
Jannink JL, Lorenz AJ, Iwata H (2010) Genomic selection in plant breeding: From theory to practice. Briefings Funct Genomics 9:166–177
Jayakodi M, Padmarasu S, Haberer G, Bonthala VS, Gundlach H et al (2020) The barley pan-genome reveals the hidden legacy of mutation breeding. Nature 588:284–289
Jia Y, Jannink JL (2012a) Multiple-trait genomic selection methods increase genetic value prediction accuracy. Genetics 192:1513–1522
Jia Y, Jannink JL (2012b) Multiple-trait genomic selection methods increase genetic value prediction accuracy. Genetics 192:1513–1522
Jiang F, Doudna JA (2017) CRISPR-Cas9 structures and mechanisms. Annu Rev Biophys 46:505–529
Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821
Kalladan R, Worch S, Rolletschek H, Harshavardhan VT, Kuntze L, Seiler C, Sreenivasulu N, Röder MS (2013) Identification of quantitative trait loci contributing to yield and seed quality parameters under terminal drought in barley advanced backcross lines. Mol Breed 32:71–90
Kiani D, Soltanloo H, Ramezanpour SS, Nasrolahnezhad Qumi AA, Yamchi A, Zaynalinezhad KH, Tavakol E (2017) A barley mutant with improved salt tolerance through ion homeostasis and ROS scavenging under salt stress. Acta Physiol Plant 39:90
Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales APW, Li Z, Peterson RT, Yeh JRJ, Aryee MJ, Joung JT (2015) Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature 523:481–485
Knox AK, Dhillon T, Cheng H, Tondelli A, Pecchioni N, Stockinger EJ (2010) *CBF* gene copy number variation at *Frost Resistance-2* is associated with levels of freezing tolerance in temperate-climate cereals. Theor Appl Genet 121:21–35
Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533:420–424
Kotak S, Larkindale J, Lee U, von Koskull-Döring P, Vierling E, Scharf K-D (2007) Complexity of the heat stress response in plants. Curr Opin Plant Biol 10:310–316
Kumar Poonia A, Kumar Mishra S, Sirohi P, Chaudhary R, Kanwar M, Germain H, Chauhan H (2020) Overexpression of wheat transcription factor (*TaHsfA6b*) provides thermotolerance in barley. Planta 252:53
Kumar S, Sachdeva S, Bhat KV, Vats S (2018) Plant responses to drought stress: physiological, biochemical and molecular basis. In: Vats S (ed) Biotic and abiotic stress tolerance in plants. Springer, Singapore, pp 1–25
Laffont JL, Wright K, Hanafi M (2013) Genotype plus genotype × block of environments biplots. Crop Sci 53:2332–2341
Lawas LMF, Zuther E, Jagadish SK, Hincha DK (2018) Molecular mechanisms of combined heat and drought stress resilience in cereals. Curr Opin Plant Biol 45:212–217
Lawrenson T, Shorinola O, Stacey N, Li C, Østergaard L et al (2015) Induction of targeted, heritable mutations in barley and *Brassica oleracea* using RNA-guided Cas9 nuclease. Genome Biol 16:258
Lorenzana RE, Bernardo R (2009) Accuracy of genotypic value predictions for marker-based selection in biparental plant populations. Theor Appl Genet 120:151–161
Lynch JP (2013) Steep, cheap and deep: An ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357

Mano Y, Takeda K (1997) Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). Euphytica 94:263–272
Manschadi AM, Hammer GL, Christopher JT, DeVoil P (2008) Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). Plant Soil 303:115–129
Marcinkowski P, Piniewski M (2018) Climate change effect on sowing and harvest dates of spring barley and maize in Poland. Intl Agrophys 32:265–271
Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO et al (2017) A chromosome conformation capture ordered sequence of the barley genome. Nature 544:427–433
McKim SM, Koppolu R, Schnurbusch T (2018) Barley inflorescence architecture. In: Stein N, Muehlbauer GJ (eds) The barley genome. Springer Intl, Cham, Switzerland, pp 171–208
Mian A, Oomen RJF, Isayenkov S, Sentenac H, Maathuis FJM, Very A-A (2011) Over-expression of an Na^+- and K^+-permeable HKT transporter in barley improves salt tolerance. Plant J 68:468–479
Michel S, Löschenberger F, Ametz C, Pachler B, Sparry E, Bürstmayr H (2019a) Simultaneous selection for grain yield and protein content in genomics-assisted wheat breeding. Theor Appl Genet 132:1745–1760
Michel S, Löschenberger F, Ametz C, Pachler B, Sparry E, Bürstmayr H (2019b) Combining grain yield, protein content and protein quality by multi-trait genomic selection in bread wheat. Theor Appl Genet 132:2767–2780
Moeinizade S, Kusmec A, Hu G, Wang L, Schnable PS (2020) Multi-trait genomic selection methods for crop improvement. Genetics 215:931–945
Moinuddin Fischer RA, Sayre KD, Reynolds MP (2005) Osmotic adjustment in wheat in relation to grain yield under water deficit environments. Agron J 97:1062–1071
Monat C, Padmarasu S, Lux T, Wicker T, Gundlach H et al (2019) TRITEX: chromosome-scale sequence assembly of *Triticeae* genomes with open-source tools. Genome Biol 20:284
Morran S, Eini O, Pyvovarenko T, Parent B, Singh R, Ismagul A, Eliby S, Shirley N, Langridge P, Lopato S (2011) Improvement of stress tolerance of wheat and barley by modulation of expression of *DREB/CBF* factors. Plant Biotechnol J 9:230–249 https://doi.org/10.1111/j.1467-7652.2010.00547.x
Munné-Bosch S, Alegre L (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. Funct Plant Biol 3:203–216
Munns R, James RA, Xu B, Athman A, Conn SJ et al (2012) Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. Nat Biotechnol 30:360–364
Muñoz-Amatriaín M, Hernandez J, Herb D, Baenziger PS, Bochard AM, et al. (2020) Perspectives on low temperature tolerance and vernalization sensitivity in barley: Prospects for facultative growth habit. Front Plant Sci 11:585927
Murelli C, Rizza F, Albini FM, Dulio A, Terzi V, Cattivelli L (1995) Metabolic changes associated with cold-acclimation in contrasting cultivars of barley. Physiol Plant 94:87–93
Muzammil S, Shrestha A, Dadshani S, Pillen K, Siddique S, Léon J, Naz AA (2018) An ancestral allele of *Pyrroline-5-carboxylate synthase1* promotes proline accumulation and drought adaptation in cultivated barley. Plant Physiol 178:771–782
Mwando E, Han Y, Angessa TT, Zhou G, Hill CB, Zhang X-Q, Li C (2020) Genome-wide association study of salinity tolerance during germination in barley (*Hordeum vulgare* L.). Front Plant Sci 11:118
Negrao S, Schmockel SM, Tester M (2017) Evaluating physiological responses of plants to salinity stress. Ann Bot 119:1–11
Neisse AC, Kirch JL, Hongyu K (2018) AMMI and GGE Biplot for genotype × environment interaction: a medoid–based hierarchical cluster analysis approach for high–dimensional data. Biometrical Lett 55:97–121
Nevo E, Brown AHD, Zohary D, Storch N, Beiles A (1981) Microgeographic edaphic differentiation of allozyme polymorphism of wild barely. Plant Syst Evol 138:287–292
Nevo E, Beiles A, Storch N, Doll H, Anderson B (1983) Microgeographic edaphic differentiation in hordein poly-morphisms of wild barley. Theor Appl Genet 64:123–132

Nevo E, Beiles A, Zohary D (1986) Genetic resources of wild barley in the Near East: structure, evolution and application in breeding. Biol J Linn Soc 27:355–380

Nishimasu H, Shi X, Ishiguro S, Gao L, Hirano S et al (2018) Engineered CRISPR-Cas9 nuclease with expanded targeting space. Science 361:1259–1262

Oliver SN, Deng W, Casao MC, Trevaskis B (2013) Low temperatures induce rapid changes in chromatin state and transcript levels of the cereal *VERNALIZATION1* gene. J Exp Bot 64:2413–2422

Orman-Ligeza B, Harwood W, Hedley PE, Hinchcliffe A, Macaulay M, Uauy C, Trafford K (2020) TRA1: a locus responsible for controlling *Agrobacterium*-mediated transformability in barley. Front Plant Sci 11:355

Peng S, Khush GS, Virk P, Tang Q, Zou Y (2008) Progress in ideotype breeding to increase rice yield potential. Field Crop Res 108:32–38

Pham A-T, Maurer A, Pillen K, Brien C, Dowling K, Berger B, Eglinton JK, March TJ (2019) Genome-wide association of barley plant growth under drought stress using a nested association mapping population. BMC Plant Biol 19:134

Pham AT, Maurer A, Pillen K, Taylor J, Coventry S, Eglinton JK, March TJ (2020) Identification of wild barley derived alleles associated with plant development in an Australian environment. Euphytica 216:148

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol 193:30–50

Reinert S, Kortz A, Léon J, Naz AA (2016) Genome-wide association mapping in the global diversity set reveals new QTL controlling root system and related shoot variation in barley. Front Plant Sci 7.1061

Rivandi J, Miyazaki J, Hrmova M, Pallotta M, Tester M, Collins NC (2011) A *SOS3* homologue maps to *HvNax4*, a barley locus controlling an environmentally sensitive Na+ exclusion trait. J Exp Bot 62:1201–1216

Rizza F, Crosatti C, Stanca AM, CattivellI L (1994) Studies for assessing the influence of hardening on cold tolerance of barley genotypes. Euphytica 75:131–138

Rizza F, Pagani D, Stanca AM, Cattivelli L (2001) Use of chlorophyll fluorescence to evaluate the cold acclimation and freezing tolerance of winter and spring oats. Plant Breed 120:389–396

Rizza F, Badeck FW, Cattivelli L, Lidestri O, Di Fonzo N, Stanca AM (2004) Use of a water stress index to identify barley genotypes adapted to rainfed and irrigated conditions. Crop Sci 44:2127–2137

Roy SJ, Negrao S, Tester M (2014) Salt resistant crop plants. Curr Opin Biotechnol 26:115

Russell J, Mascher M, Dawson IK, KyriakidisS Calixto C et al (2016) Exome sequencing of geographically diverse barley landraces and wild relatives gives insights into environmental adaptation. Nat Genet 48:1024–1030

Saade S, Maurer A, Shahid M, Oakey H, Schmöckel SM, 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 6:32586

Saade S, Brien C, Pailles Y, Berger B, Shahid M et al (2020) Dissecting new genetic components of salinity tolerance in two-row spring barley at the vegetative and reproductive stages. PLoS One 15:e0236037

Saoudi W, Badri M, Taamalli W, Zribi OT, Gandour M, Abdelly C (2019) Variability in response to salinity stress in natural Tunisian populations of *Hordeum marinum* subsp. *marinum*. Plant Biol 21:89–100

Schilling RK, Marschner P, Shavrukov Y, Berger B, Tester M, Roy SJ, Plett DC (2014) Expression of the Arabidopsis vacuolar H+-pyrophosphatase gene (*AVP1*) improves the shoot biomass of transgenic barley and increases grain yield in a saline field. Plant Biotechnol J 12:78–386

Schmalenbach I, Körber N, Pillen K (2008) Selecting a set of wild barley introgression lines and verification of QTL effects for resistance to powdery mildew and leaf rust. Theor Appl Genet 117:1093–1106

Schreiber M, Mascher M, Wright J, Padmarasu S, Himmelbach A, Heavens D, Milne L, Clavijo BJ, Stein N, Waugh R (2020) A genome assembly of the barley 'transformation reference' cultivar Golden Promise. G3 Genes Genomics Genet 10:1823–1827

Shamsudin NAA, Swamy BPM, Ratnam W, Cruz MTS, Sandhu N, Raman AK, Kumar A (2016) Pyramiding of drought yield QTLs into a high quality Malaysian rice cultivar MRQ74 improves yield under reproductive stage drought. Rice 9:21

Shavrukov Y, Gupta NK, Miyazaki J, Baho MN, Chalmers KJ, Tester M, Langridge P, Collins NC (2010) *HvNax3* a locus controlling shoot sodium exclusion derived from wild barley (*Hordeum vulgare* ssp. *spontaneum*). Funct Integr Genet 10:277–291

Shavrukov Y, Bovill J, Afzal I, Hayes JE, Roy SJ, Tester M, Collins NC (2013) *HVP10* encoding V-PPase is a prime candidate for the barley *HvNax3* sodium exclusion gene: evidence from fine mapping and expression analysis. Planta 237:1111–1122

Shavrukov Y, Kurishbayev A, Jatayev S, Shvidchenko V, Zotova L, Koekemoer F, de Groot S, Soole K, Langridge P (2017) Early flowering as a drought escape mechanism in plants: how can it aid wheat production? Front Plant Sci 8:1950

Shen Q, Yu J, Fu L, Wu L, Dai F, Jiang L, Wu D, Zhang G (2018) Ionomic, metabolomic and proteomic analyses reveal molecular mechanisms of root adaption to salt stress in Tibetan wild barley. Plant Physiol Biochem 123:319–330

Sivamani E, Bahieldin A, Wraith JM, Al-Niemi T, Dyer WE, Ho T-HD, Qu R (2000) Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley *HVA1* gene. Plant Sci 155:1–9

Skinner JS, von Zitzewitz J, Szucs P, Marquez-Cedillo L, Filichkin T et al (2005) Structural, functional, and phylogenetic characterization of a large *CBF* gene family in barley. Plant Mol Biol 59:533–551

Skinner JS, Szűcs P, von Zitzewitz J, Marquez-Cedillo L, Filichkin T, Stockinger EJ, Thomashow MF, Chen THH, Hayes PM (2006) Mapping of barley homologs to genes that regulate low temperature tolerance in Arabidopsis. Theor Appl Genet 112:832–842

Soltesz A, Smedley M, Vashegyi I, Galiba G, Harwood W, Vaguifalvi A (2013) Transgenic barley lines prove the involvement of *TaCBF14* and *TaCBF15* in the cold acclimation process and in frost tolerance. J Exp Bot 64:1849–1862

Song G, Zhang R, Zhang S, Li Y, Gao J, Han X, Chen M, Wang J, Li W, Li G (2017) Response of microRNAs to cold treatment in the young spikes of common wheat BMC. Genomics 18:212

Stockinger EJ, Skinner JS, Gardner KG, 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. Plant J 51:308–321

Subburaj S, Tu L, Jin Y-T, Bae S, Seo PJ, Jung YJ, Lee G-J (2016) Targeted genome editing, an alternative tool for trait improvement in horticultural crops. Hortic Environ Biotechnol 57:531–543

Tang X, Lowder LG, Zhang T, Malzahn AA, Zheng X, Voytas DF, Zhong Z, Chen Y, Ren Q, Li Q, Kirkland ER, Zhang Y, Qi Y (2017) A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants. Nat Plants 3:17018

Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot 49:419–432

Tardieu F, Tuberosa R (2010) Dissection and modelling of abiotic stress tolerance in plants. Curr Opin Plant Biol 13:206–212

Tardieu F, Sionneau T, Muller B (2018) The physiological basis of drought tolerance in crop plants: a scenario-dependent probabilistic approach. Annu Rev Plant Biol 69:733–759

Tavakkoli E, Fatehi F, Coventry S, Rengasamy P, McDonald GK (2011) Additive effects of Na^+ and Cl^- ions on barley growth under salinity stress. J Exp Bot 62:2189–2203

Tinker N, Yan W, Tinker NA (2015) Biplot analysis of multi-environment trial data: Principles and applications Biplot analysis of multi-environment trial data: principles and applications. Can J Plant Sci 6:623–644

Tondelli A, Francia E, Barabaschi D, Pasquariello M, Pecchioni N (2011) Inside the *CBF* locus in Poaceae. Plant Sci 180:39–45

Tondelli A, Pagani D, Naseh Ghafoori I, Rahimi M, Ataei R, Rizza F, Flavell AJ, Cattivelli L (2014) Allelic variation at *Fr-H1/Vrn-H1* and *Fr-H2* loci is the main determinant of frost tolerance in spring barley. Environ Exp Bot 106:148–155

Tondelli A, Crosatti C, Delbono S, Cattivelli L (2020) Advances in understanding of barley plant physiology: response to abiotic stress. In: Fox G, Li C (eds) Achieving sustainable cultivation of barley. Burleigh Dodds Science Publishing, Cambridge, UK. ISBN-13:9781786763082

Turner NC (2004) Sustainable production of crops and pastures under drought in a Mediterranean environment. Ann Appl Biol 144:139–147

Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310:1031–1034

Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet 45:1097–1102

van Raden PM (2008) Efficient methods to compute genomic predictions. J Dairy Sci 91:4414–4423

von Korff M, Wang H, Léon J, Pillen K (2006) AB-QTL analysis in spring barley. II. Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (*H. vulgare* ssp. *spontaneum*). Theor Appl Genet 112:1221–1231

von Zitzewitz J, Szucs P, Dubcovsky J, Yan L, Francia E, Pecchioni N, Casas A, Chen THH, Hayes PM, Skinner JS (2005) Molecular and structural characterization of barley vernalization genes. Plant Mol Biol 59:449–467

von Zitzewitz J, Cuesta-Marcos A, Condon F, Castro AJ, Chao S et al (2011) The genetics of winterhardiness in barley: perspectives from genome-wide association mapping. Plant Genome 4:76–91

Voss-Fels KP, Robinson H, Mudge SR, Richard C, Newman S et al (2018) *VERNALIZATION1* modulates root system architecture in wheat and barley. Mol Plant 11:226–229

Wallwork MAB, Jenner CF, Logue SJ, Sedgley M (1998) Effect of high temperature during grain-filling on the structure of developing and malted barley grains. Ann Bot 82:587–599

Wang M, Mao Y, Lu Y, Tao X, Zhu J-K (2017) Multiplex gene editing in rice using the CRISPR-Cpf1 system. Mol Plant 10:1011–1013

Wardlaw IF, Wrigley CW (1994) Heat tolerance in temperate cereals: an overview. Aust J Plant Physiol 21:695–703

Wiegmann M, Maurer A, Pham A, March TJ, Al-Abdallat A et al (2019) Barley yield formation under abiotic stress depends on the interplay between flowering time genes and environmental cues. Sci Rep 9:6397

Wu H, Shabala L, Zhou M, Stefano G, Pandolfi C et al (2015a) Developing and validating a high-throughput assay for salinity tissue tolerance in wheat and barley. Planta 242:847–857

Wu H, Zhu M, Shabala L, Zhou M, Shabala S (2015b) K^+ retention in leaf mesophyll, an overlooked component of salinity tolerance mechanism: a case study for barley. J Integr Plant Biol 57:171–185

Xia Y, Li R, Ning Z, Bai G, Siddique KHM, Yan G, Baum M, Varshney RK, Guo P (2013) Single nucleotide polymorphisms in HSP17.8 and their association with agronomic traits in barley. PLoS One 8:e56816

Xie W, Xiong W, Pan J, Ali T, Cui Q, Guan D, Meng J, Mueller ND, Lin E, Davis SJ (2018) Decreases in global beer supply due to extreme drought and heat. Nat Plants 4:964–973

Xu S, Chong K (2018) Remembering winter through vernalisation. Nat Plants 4:997–1009

Xue GP, Loveridge CW (2004) *HvDRF1* is involved in abscisic acid-mediated gene regulation in barley and produces two forms of AP2 transcriptional activators, interacting preferably with a CT-rich element. Plant J 37:326–339

Xue W, Yan J, Zhao G, Jiang Y, Cheng J, Cattivelli L, Tondelli A (2017) A major QTL on chromosome 7HS controls the response of barley seedling to salt stress in the Nure × Tremois population. BMC Genet 18:79

Xue W, Jiang Y, Zhan Z, Yan J, Cattivelli L, Tondelli A, Cheng J (2019) Genetic dissection of winter barley seedling response to salt and osmotic stress. Mol Breed 39:137

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yoo CY, Pence HE, Jin JB, Miura K, Gosney MJ, Hasegawa PM, Mickelbart MV(2010) The Arabidopsis *GTL1* transcription factor regulates water use efficiency and drought tolerance by modulating stomatal density via trans-repression of SDD1. Plant Cell 22:4128–4141

Zhang Y, Liang Z, Zong Y, Wang Y, Liu J et al (2016) Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat Commun 7:12617

Zhang X, Shabala S, Koutoulis A, Shabala L, Zhou M (2017) Meta-analysis of major QTL for abiotic stress tolerance in barley and implications for barley breeding. Planta 245:283–295

Zhang M, Fu M-M, Qiu C-W, Cao F, Chen Z-H, Zhang G, Wu F (2019) Response of Tibetan wild barley genotypes to drought stress and identification of quantitative trait loci by genome-wide association analysis. Intl J Mol Sci 20:791. https://doi.org/10.3390/ijms20030791

Zhu JK (2016) Abiotic stress signalling and responses in plants. Cell 167:313–324

Zhu M, Zhou MX, Shabala L, Shabala S (2015) Linking osmotic adjustment and stomatal characteristics with salinity stress tolerance in contrasting barley accessions. Funct Plant Biol 42:252–263

Zhu M, Zhou MX, Shabala L, Shabala S (2017) Physiological and molecular mechanisms mediating xylem Na^+ loading in barley in the context of salinity stress tolerance Plant Cell Environ 40:1009–1020

Chapter 5
Advances in Genomic Designing for Abiotic Stress Tolerance in Sorghum

B. Fakrudin, T. N. Lakshmidevamma, J. Ugalat, J. Khan, S. P. Gautham Suresh, K. A. Apoorva, M. Doddamani, S. Kadam, K. Omkar Babu, A. Hadimani, M. N. Mamathashree, K. Rashmi, M. Faizan, A. Daspute, Prakash Gopalareddy, Aurag Gowda, and Raghavendra Gunnaiah

Abstract Sorghum (*Sorghum bicolor* (L.) Moench) is a multipurpose C_4 plant that ranks fifth among the important cereal crops at the global level. It is a staple crop well-adapted to water-deficit environments, hence, considered as a crop of choice for marginal lands. Inherently sorghum possesses high levels of abiotic stress tolerance compared to other cereal crops. Sorghum, however, has various morphological, anatomical, physiological responses and many molecular signal network mechanisms in imparting abiotic stress tolerance. The major challenging area of research in the climate change scenario is to develop and deploy resistant varieties. Researchers have been trying to decode the complex phenomenon of sorghum tolerance to abiotic stress using various approaches. The extent of crop yield loss due to abiotic stresses can be effectively addressed by combining conventional breeding with high throughput omics technologies. This chapter highlights the broader view of abiotic stress factors and their influence on the sorghum yield. The underlying mechanisms at various levels adapted by sorghum are covered in this chapter. This chapter also describes breeding principles utilized to obtain abiotic stress-tolerant sorghum varieties. A glimpse of genomics aided breeding coupled with conventional breeding methodologies are expected to provide a promising

B. Fakrudin (✉) · T. N. Lakshmidevamma · J. Ugalat · S. P. Gautham Suresh · K. A. Apoorva · M. Doddamani · S. Kadam · A. Hadimani · M. N. Mamathashree · K. Rashmi · M. Faizan · P. Gopalareddy · A. Gowda · R. Gunnaiah
Department of Biotechnology and Crop Improvement, College of Horticulture, University of Horticultural Sciences, Campus, Bengaluru 560065, India
e-mail: bfakrudin@uhsbagalkot.edu.in

J. Khan
Center for Cellular and Molecular Platforms, National Center for Biological Sciences, Bengaluru 560065, India

K. Omkar Babu
Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

A. Daspute
College of Agricultural Biotechnology, Ahmednagar, Maharashtra, India

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approach in improving crop productivity. This chapter provides readers an overview of modern omics era covering sequencing, gene network elucidation through transcriptomics, validation through expression profiling, editing that may aid in sorghum improvement.

Keywords Sorghum · Multipurpose · Abiotic stresses · Drought · Transcriptomics

5.1 Introduction

Sorghum, (*Sorghum bicolor* (L.) Moench) is a multipurpose C_4 plant that ranks fifth among the most important cereal crops. This is an ancient grain crop with broad adaptation, originated and domesticated in Africa (Pontieri et al. 2016). It is a staple crop for an estimated half a billion people as food, feed, fuel and fiber (Mace et al. 2013; Boyles et al. 2019). This crop is used in several industrial applications including production of bioethanol and alcoholic beverages (Venkateswaran et al. 2019). Sorghum is rather an unrecognized nutrient-rich cereal grain in the human diet. Uncooked sorghum grains of 100 g could provide 316 kilocalories of energy, 69 g of carbohydrates, proteins (10 g), fat (3 g), fiber (6 g) and vitamin B complex and minerals such as copper, iron, phosphorous, etc. with numerous added health benefits (Rao et al. 2014).

Sorghum is well-adapted to water-deficit environments, hence, considered as a crop of choice for marginal lands. Inherently sorghum possesses high levels of drought and heat tolerance compared to other cereal crops (Tuinstra et al. 1997; Hall et al. 2004). Several heritable morphological, anatomical, physiological responses and many adaptive mechanisms are involved in imparting drought tolerance in sorghum (Krupa et al. 2017). Several sorghum cultivars and hybrids grown today for high grain yield, earliness, drought adaptation and ethanol production are the results of intensive selections (Evans et al. 2013). Sorghum is a good crop model to study the genotype-phenotype association as well as to dissect genotype and environment (G X E) interactions (Abdel-Ghany et al. 2020). Further, the fully sequenced genome with ~730 Mbp makes it an attractive model for studying functional genomics of abiotic stresses in cereal crop plants (Paterson et al. 2009).

The genus Sorghum with 25 diverse species belongs to the family Poaceae and the tribe, Andropogoneae (Bonnett and Henry 2011; Anzoua et al. 2011). Five taxonomic subgenera or sections viz., Eu-sorghum, Heterosorghum, Chaetosorghum, Stiposorghum and Para-Sorghum are delineated in the genus sorghum (Dillon et al. 2007). The cultivated species, *Sorghum bicolor* (L.) (2n = 20) is believed to have descended from its wild progenitor *S. bicolor* subsp. v*erticilliflorum*. Five morphological sub-species or races of *S. bicolor* and their intermediates are known based on panicle structure: *bicolor, caudatum, durra,*

guinea and kafir. Sorghum is predominantly a self-pollinating but may show up to 50% cross-pollination, which has aided in its evolution and rapid adaptation to tropical and subtropical regions (Osuna-Ortega et al. 2003). The rich genetic diversity and crop wild relatives (CWR) in the genus Sorghum could serve as sources of new genes for addressing the multiple man-made problems including climate change (Dillon et al. 2007).

5.1.1 Reduction in Yield Due to Abiotic Stresses

Abiotic stresses are considered as the most devastating environmental stresses that limit and hinder the productivity of sorghum. However, sorghum can withstand prolonged dry periods due to inherent tolerance to abiotic stresses in comparison with other cereal crops (Tuinstra et al. 1997). Even after prolonged drought stress, the crop can resume its growth once the soil moisture is available. Sorghum is largely grown in areas that receive annual rainfall between 350 and 700 mm (Mundia et al. 2019). Since it is primarily a rain-fed crop, its yield levels mainly depend on its inherent drought resistance and the crop requires optimum soil moisture between 25 and 50% field capacity (Fawusi and Agboola 1980). At present, altered precipitation patterns, possibly owing to climate change eventualities, are the major limiting factors (Akinseye et al. 2020). Consequently, severe abiotic stress factors such as drought, heat and salinity cause major hindrance to the cultivation. Yield and biomass losses of up to 90% under extreme stress conditions were reported (House 1985). The greater impact of drought stress in sorghum was during grain-filling stage which results in plant senescence, premature leaf death, stalk lodging, and charcoal rot with poor seed and stover yield (Akman et al. 2020). Though it is a heat-loving crop, sorghum cannot withstand high temperatures beyond 35 °C. The high sensitivity of sorghum to water stress from floret differentiation to early bloom was also indicated (Prasad et al. 2015). A reduction of 28% and 30% in seed number and yield, respectively, was recorded when the temperature of 5 °C above ambient prevailed for around 2–3 weeks at the floret differentiation stage (Dayakar Rao et al. 2004). Low temperatures, especially below-freezing temperatures, render sorghum to grow very slowly or die (Petsakos et al. 2019). Plant vigor and yields are significantly reduced due to aluminium toxicity.

5.1.2 Growing Importance in the Face of Climate Change and Increasing Population

In the United States of America (USA), Australia, Mexico, Argentina and China, sorghum is produced on a profitable scale, predominantly for export as grain and for biofuel production. Although sorghum is cultivated on marginal lands in these

developed countries, with the use of improved varieties, hybrid seeds and modern farming practices, relatively high grain yield levels of four tons per hectare have been achieved (Singh et al. 2014). However, if cultivated with better management under optimal conditions, it can produce a record yield of 20 tons per hectare for grain and biomass of 80 tons (Singh et al. 2014). Further, the countries like India, Sudan, Nigeria and other African countries, where sorghum is primarily cultivated for human consumption in low-input farming systems have recorded on an average yield level as low as one ton per hectare (Rakshit et al. 2014).

The global demand for sorghum has a potential to rise due to several factors such as its use as a low-cost alternative for corn as livestock feed, health benefits due to its gluten-free nature and other medicinal properties, drought tolerance, and scope for yield improvement as the current global yield is below its potential (Mundia et al. 2019). However, the crop has been neglected to the status of an orphan crop. The progress in yield enhancements is relatively low in developed countries and even a declining trend in Nigeria and Sudan was noticed (Taylor and Duodu 2018).

The fluctuating climate accompanied by increased heat stress, floods and drought are affecting crop yields and biomass production. So the climate-smart sorghum is a preferred crop owing to its adaptation and yielding ability under harsh environments (Kim 2003). The dense and deep root system and ability to lower the metabolic processes during terminal stress besides reduction in transpiration through the stomatal closure and leaf rolling are considered as the possible mechanisms of drought tolerance in sorghum (Schittenhelm and Schroetter 2014; Hadebe et al. 2017). In addition, under the present climate change scenario, climate change has a positive impact on sorghum production attributed to its much higher tolerance to drought than corn because of its ability to take up water and hold more efficiently that is attributed to its smaller leaf to root ratio with the extensive root system and heavy wax layer. These factors may lead to increased production in regions that are experiencing extreme drought as a result of climate change (Akinseye et al. 2020).

5.1.3 Limitations of Traditional Breeding and Rational of Genome Designing

Crop improvement efforts so far have contributed tremendously to develop high yielding varieties and hybrids. Breeding for abiotic stress tolerance, especially moisture stress tolerance is a major challenging area of research in the climate change scenario. Development of photoperiod-sensitive and insensitive lines and hybrids was possible through population improvement programs (Rooney and Aydin 1999). Moisture stress tolerance has been observed both in stabilised genotypes and hybrids of sorghum (Khatab et al. 2017). Drought tolerance index in five sorghum genotypes under contrasting moisture regimes was assessed:

Taggat-14 genotype performed the best (Jabereldar et al. 2017). Lack of knowledge on the molecular pathways concerning abiotic stress in sorghum has been a limiting factor for their effective management (Foolad, 2007; Collard and Mackill 2008). Advances in screening and breeding for the stay-green trait at terminal stages of the crop is considered a key approach to develop drought tolerance in sorghum (Subudhi et al. 2000). Deployment of advanced breeding techniques based on molecular genetics and genomics could accelerate plant breeding. Since the gene network involved in abiotic stress tolerance is complex, the genetic dissection of associated genomic regions can be achieved through the quantitative trait locus (QTL) mapping approaches (Collard et al. 2005).

5.2 Physiological and Molecular Mechanism of Abiotic Stress Tolerance

Plant adaptation to abiotic stresses is strictly attributed to molecular signalling networks and change in the metabolite content in different plant parts (Chinnusamy et al. 2004). Adaptive stress tolerance mechanisms involve hormonal balance adjustment, synthesis of stress-related proteins and enzymes, activation of antioxidant defense mechanism for detoxifying the reactive oxygen species, reorganization of the metabolite accumulation, and restructuring of the cellular membrane (Khan et al. 2020). Signal transduction networks involved in abiotic stresses are of three types: (a) osmotic/oxidative stress signalling that uses protein kinase modules, involves the accumulation of reactive oxygen species (ROS) scavenging antioxidant systems, as well as osmolytes, (b) Ca^{2+} dependent signalling that leads to the activation of late embryogenesis abundant (LEA)-type genes, involves the production of stress-responsive proteins mostly of undefined functions and (c) Ca^{2+} reliant salt overly sensitive (SOS) signalling that regulates ion. Stress resistance traits, maintain cellular hydrostatic pressure, by physiological modifications, mainly through osmotic adjustments (Blum 2017). Physiological, morphological and phenological level reactions are displayed by sorghum (Verma et al. 2018) (Fig. 5.1).

Drought-tolerant varieties have exhibited the high-water use efficiency (WUE), extensive root system and thick waxy cuticle to avoid excessive transpiration (Mayaki et al. 1976; Jordan 1980). These were also noted for the accumulation of varying amounts of osmolytes to balance the osmotic potential of the cell and maintain the homeostasis (Newton et al. 1986; Wood et al. 1996; Devnarain et al. 2016). Sometimes, late-flowering cultivars can tolerate water deficit more effectively (Hsiao et al. 1976). When plants sense a decrease in soil water content, they activate the biosynthesis and accumulation of abscisic acid (ABA). ABA, in turn, activates stomatal closure and the expression of stress-inducible genes. ABA seems to regulate the rate of transpiration and activates genes that encode proteins having diverse functions concerning drought tolerance (Bray 1993; Seki et al. 2003).

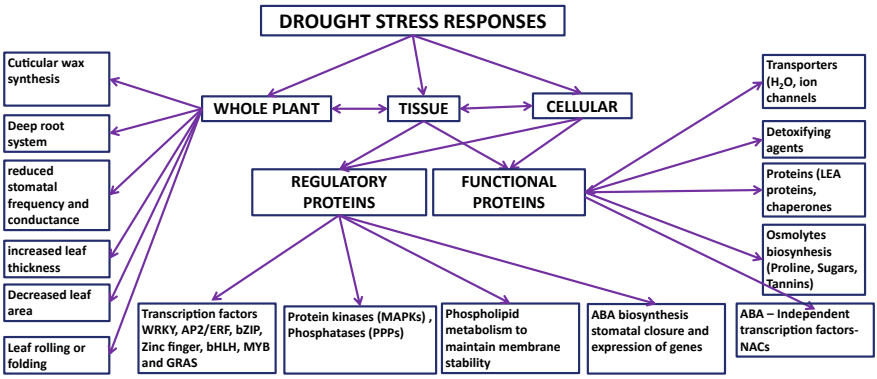

Fig. 5.1 Drought stress responses at whole plant, tissue and cellular level

Immediate upregulated drought stress-responsive genes include heat shock proteins (e.g., HSP and chaperonins), response to ABA, response to ROS/oxidative stress and programmed cell death. Different response profiles of metabolites, heat shock proteins (HSPs) and dehydrins (DHNs) were observed in the two cultivars (Ogbaga et al. 2016).

Products of the expressed genes are the drought-induced proteins such as late embryogenesis abundant proteins (LEA), chaperones and ROS detoxification enzymes that directly protect cellular components against oxidative osmotic stress-induced damage. Other proteins, such as transcription factors (TFs), protein kinases and phospholipase C, have gene regulatory and signalling functions (Shinozaki and Yamaguchi-Shinozaki 1996). Many differentially expressed genes (DEGs) have been elucidated in sorghum through a transcriptomics approach. Top upregulated genes are the genes coding for LEA proteins, water stress-induced WSI18 protein and dehydrins (Dugas et al. 2011). Several novel genes besides many transcription factors (TFs) and stress-related proteins have been implicated in drought tolerance in sorghum (Abdel-Ghany et al. 2020).

5.3 Drought Stress in Sorghum

Drought is the major abiotic stress that adversely affects plant growth and productivity. It is a complex phenomenon whose expression in crop plants is mainly dependent on morphological (earliness, reduced leaf area, leaf rolling, efficient rooting system and stability of yield) and biochemical (accumulation of proline, betaine) parameters and at the molecular level, drought being regulated by both functional and regulatory sets of genes (Bartels and Sunkar 2005). Sorghum is an annual, warm-season grain crop, which can withstand abiotic stresses compared to most other cereal crops (Hall 2000).

Moisture stress hinders multiple factors involved in the process of seed germination including the growth of mesocotyl, radicle and shoot of sorghum (Blum 1996; Bayu et al. 2005). Moisture stress during vegetative growth reduces the rate of cell expansion, cell size and consequently, growth rate, stem elongation, and leaf expansion (Garrity et al. 1984; Hale and Orcutt 1987; Khaton et al. 2016). The detrimental effects of moisture stress on the number of ear heads, crop stand, number of seeds per head, tillering capacity and yield are evident (Thomas and Howarth 2000; Xin et al. 2008). The reproductive growth stage is more susceptible to moisture stress compared to others (Kramer 1983). Among the reproductive related traits development of pollen, ovules and fertilization process are severely affected due to moisture stress in sorghum. Moisture stress during harvesting stage results in reduced grain yield through its component traits such as the reduction in the number of grains per panicle, test weight and the number of filled grain per hill (Khaton et al. 2016).

Genetic variability for drought-tolerant and susceptible cultivars at different developmental stages of sorghum facilitates global analysis of gene expression to identify drought-regulated genes specific to tolerant genotypes. Drought-related genes in QTLs encode important regulatory proteins that are related to drought stress. The availability of the sorghum genome sequences and global gene expression data using either microarray or RNA-Seq are elucidating the genes that contribute to the stress tolerance in sorghum (Paterson et al. 2009; Dugas et al. 2011; Fracasso et al. 2016). The open reading frames of quantitative trait loci (QTLs) are increasingly being elucidated (Mace and Jordan 2011; Fakrudin et al. 2013; Harris-Shultz et al. 2019). Discerning the genomic regions for root related QTLs can facilitate improvement in sorghum yield through genomics-assisted breeding (GAB). Further, leaf rolling, a typical drought avoidance mechanism in sorghum was found to be regulated by major genes including *RL7, RL8*, and *RL9* and a single recessive allele of each gene revealed rolled leaves (Zhang et al. 2019).

5.3.1 Leaf Stay—Green Trait

Stay-green is a post-flowering abiotic resistance trait that stabilises the yield under moisture stress regime in some plants (Tao et al. 2000; Borrell et al. 2014). Post-flowering stay-green of the leaf biomass is a pronounced and relatively well-characterised trait in post-rainy sorghum genotypes (Pandey et al. 2018). Stay-green QTLs impart sustenance of leaf biomass during the grain filling stage (Vadez et al. 2011; Jordan et al. 2012). Sorghum genotypes with this trait not only survive scanty soil moisture, but also can resume growth when congenial conditions prevail (Pandey et al. 2018). Further, genetic analysis showed that QTLs for high temperature and drought tolerance coincide with loci for leaf senescence (Vijayalakshmi et al. 2010; Jordan et al. 2012; Emebiri 2013). The ability of leaves to stay green in terminal stages of growth in sorghum is controlled by a major gene with dominant gene action (Walulu et al. 1994).

Flowering time, sink strength and variation in the day and night temperatures besides relative humidity directly influence the expression of the stay-green trait (Krupa et al. 2017). Molecular mapping efforts have revealed three major stay-green QTLs viz., *StgA, StgD*, and *StgG* that contributed to 42 per cent of the phenotypic variability and a set of other four minor QTLs viz., *StgB, StgI.1, StgI.2*, and *StgJ* (Xu et al. 2000). QTLs for nodal root angle (*qRA1_5, qRA2_5, qRA1_8, qRA1_10*) and three QTLs for root dry weight (*qRDW1_2, qRDW1_5, qRDW1_8*) have been mapped in sorghum (Fakrudin et al. 2013). Two sorghum recombinant inbred line populations (RILs) were developed from crosses IS9830 × E36-1 and N13 × E36-1 to map the stay-green QTLs (Haussmann et al. 2002). Further, field evaluation of three stay-green QTLs introgressed near-isogenic lines (NILs) revealed genetic basis in post-rainy sorghum (Chaudhari and Fakrudin 2017). QTLomic analysis of stay-green QTLs has pointed at involvement of specific *TF* genes in manifesting stay-green trait (Fakrudin et al. 2013). The genetic basis of the complex stay-green trait was dissected by fine-mapping of RSG04008-6 (stay-green) × J2614-11 (moderately senescent). APETALA2 (AP2)/ERF proteins (Sobic.010G202700), NBS-LRR protein (Sobic.010G205600), ankyrin-repeat protein (Sobic.010G205800), senescence-associated protein (Sobic.010G270300), WD40 (Sobic.010G205900), CPK1 adapter protein (Sobic.010G264400) and LEA2 protein (Sobic.010G259200) have been implicated in drought tolerance in sorghum (Kiranmayee et al. 2020).

5.4 Salinity Stress

Saline soils are a growing problem in the agricultural landscapes of the world (Landi et al. 2017). As per an estimate, over 621,597 sq km of the soils worldwide are affected by salinity. The percentage of the salt-affected soils are increasing all over the world and it is estimated that 19.5% of all irrigated land and 2.1% of dry land is affected by salt stress (Rajabi Dehnavi et al. 2020). The vital metabolic processes in the plants such as photosynthesis, protein synthesis, and lipid metabolism are affected which are major factors that determine the growth and yield (Sui et al. 2010; Sui and Han 2014; Sui et al. 2018). Survival of the limited number of plants in saline soils can lead to desertification (Flowers et al. 2010). The irrigated systems are mostly affected by the continued salinization of arable land and thus posing an increased threat to global crop production (Flowers et al. 2010). Better crop yields through eco-friendly, cost-effective and sustainable approaches would be possible with enhanced inherent salinity tolerance in crop plants (Flowers et al. 2010). Besides the plant species in question, factors such as salt composition, salt concentration, and the physiological stage of the plant would determine the extent of plant growth retardation under varying salt levels (Swami et al. 2011).

Several adaptive mechanisms for salt stress are present within the plants which occur at multiple levels and manifested at the molecular, physiological and biochemical levels (Leng et al. 2018). Differential expression of genes occurs after

plants sense the external salt-stress signals followed by overexpression of specific genes (Zhu 2001; Pang et al. 2011; Cao et al. 2017). Genes such as sucrose nonfermenting-1-related kinases (*SnRK*) and salt-overly sensitive3 (*SOS3*) genes are well known to perform the role in sensing and signalling in roots (Munns and Tester 2008). The photosynthetic response genes include enhanced response to ABA1 (*ERA1*), protein phosphatase 2C (*PP2C*), abscisic acid-activated protein kinase (*AAPK*), and phytochrome kinase substrate-like protein (*PKS3*). Candidate genes identified for salinity tolerance include genes linked with osmotic and ionic stress. In sorghum, the regulation of the vital metabolic processes for the salt stress would ameliorate the salt tolerance (Yang et al. 2020). It is found that the sugar content of the sweet sorghum increases in saline lands thus regarded as a potential source for identifying salt-related genes (Yang et al. 2020). QTL analysis in a recombinant inbred line population resulted in detection of 53 QTLs for the six traits corresponding salt tolerance index (Wang et al. 2020).

5.5 Heat Stress

The upsurge in temperature around the world is considered as a prime factor for the decreased yields of crops by causing irreversible damage to plant growth and developmental stages (Djanaguiraman et al. 2018; Ortiz-Bobea et al. 2019). Sorghum has a mechanism to avoid the heat during the pollination process by regulating its anther dehiscence and releasing pollen from in the early morning hours (Rhodes et al. 2017). However, like many other crops sorghum is more sensitive to heat stress during its reproductive stage compared to the vegetative stage (Djanaguiraman et al. 2018). Among the reproductive stages, flowering and 5–10 days before flowering are considered as the most sensitive stages (Prasad et al. 2015). Heat stress (40/30 °C) during the reproductive stage increased stomatal conductance and transpiration rate by 19.9% and 17.4%, respectively, under controlled environment conditions (Prasad et al. 2008; Djanaguiraman et al. 2010). Heat stress during grain-filling decreases the grain-filling duration and rate resulting in reduced individual grain weight per plant but doesn't affect seed-setting and grain number (Prasad et al. 2015; Singh et al. 2015).

Pathways, genes and processes backing heat stress tolerance could be effectively realized through transcriptomic analyses of plant responses to stress. Seedling heat stress traits were associated with 12 single nucleotide polymorphisms (SNPs), that function in ion transport pathways and sugar metabolism functions (Chopra et al. 2017). Sorghum exposed to more than 36–38 °C for 10–15 days during the flowering time resulted in reduced pollen germination. Further, the planting date and temperature significantly affected the seed production of sorghum (Angarawai et al. 2017). Elevated tolerance to high temperature is expected to help in enhancing sorghum productivity (Chiluwal et al. 2020).

5.6 Cold Stress

In comparison with other cereals, a crucial factor that limits temperate sorghum production is susceptibility to cold stress. It decreases seed germination, seedling vigor and seedling emergence. The germination of seed and plant emergence scale down when the temperature falls below 15 °C, which in turn lower plant density; a character that is considered as a vital component of crop yield (Maulana et al. 2017). The germplasm owing its origin in China manifested greater cold tolerance, in contrast with many other sorghums, descending from the tropical semi-arid regimes (Franks et al. 2006). Cold tolerant standard check Shan Qui Red under-performed or showed marginally better tolerance when compared to native breeds of sorghum from the temperate regions, this was attributed to the seedling vigour (Maulana et al. 2017). General combining ability (GCA) seems to be more than specific combining ability (SCA) for cold tolerance. Hence, crosses made among lines with high GCA effects will have hybrids/base population with the best cold tolerance (Yu and Tuinstra 2001).

A QTL linked to early-season performance under both cold and optimal conditions was discerned from a RIL population, derived from the cold-tolerant Chinese line, 'Shan Qui Red' (SQR). For germination under cold stress and optimal environmental conditions, two associated QTLs were identified on the linkage group SBI-03a and another on SBI-07b that denoted greater significance. Composite interval mapping (CIM) was employed on SBI-02 for identifying a QTL for both early as well as late emergence (Knoll and Ejeta 2008). QTLs from cold-tolerant cultivar PI610727 associated with cold germinability and for field emergence were discerned and a promising line- Fearlygerm-9.3, was derived from RILs of RTx430 and PI610727 (Burow et al. 2011). Some interactive epistatic QTL hotspots accountable for cold stress tolerance were also identified (Bekele et al. 2014). Several genes conferring growth and maintenance of cell division under early chilling stress within QTL hotspot regions were elucidated from candidate gene network within QTL regions. A low-temperature germination marker locus (*Locus 7-2*) has been identified and it revealed a significant association with the trait (Upadhyaya et al. 2016). The extensive disparity for seedling traits under cold and heat stress was sighted in the sorghum association panel. Tagged genes that are associated with soluble carbohydrate metabolism and regulation of anthocyanin expression and traits were identified under cold stress using 30 SNPs through genome-wide association studies (GWAS). A nested association mapping (NAM) population was utilized to anatomize the genetic construction of cold tolerance in early season sorghum. Cold tolerance mechanism in contrasting cultivars of sorghum was elucidated using omics aided in silico analysis. A set of 1910 differentially expressed genes was revealed under cold stress. The key transcription factors such as dehydration responsive element binding (DREB), ethylene-responsive, and C-repeat binding factors were found to be upregulated under cold stress. A total of 41,603 SNPs were utilised to map cold tolerance related QTLs and associated transcription factors in sorghum (Marla et al. 2017).

5.7 Waterlogging

Among the various abiotic constraints in sorghum production, waterlogging stress curtails the yield levels significantly which necessitates a better perception and realization of plant responses to waterlogged soils. Waterlogging has a particularly deleterious effect through the direct influence on plant metabolism and by creating unfavourable changes in the soil physical properties such as structure and texture (Orchard and Jessop 1984). Although the effects of flooding depend on the age of the crop, sensitivity to flooding generally results in the highest reduction in dry matter accumulation (Promkhambut et al. 2011). However, waterlogging to sorghum crop for 30 days or more did not significantly affect shoot growth: this trait exhibits high genetic variation among the sorghum genotypes (Pardales et al. 1991; Promkhambut et al. 2011). New nodal root development, ability to produce new leaves and increased root length are considered as adaptive responses of sorghum to waterlogging (Promkhambut et al. 2010). The inherent biochemical mechanism responsible for adaptation to waterlogged situations is the fermentative metabolism under anaerobiosis. Enzymes such as alcohol dehydrogenase and lactate dehydrogenase are directly involved in the process as reflected in the flood-tolerant sorghum variety (Jain et al. 2010). More specifically, a progressive increase in the activity of phosphofructokinase, pyruvate kinase and fructose-1,6-bisphosphate aldolase (FBP aldolase) was observed in the roots of flood-tolerant genotypes compared to rather a slight change in the activity of FBP aldolase in floor-sensitive genotypes (Singla et al. 2003). Leaf area (69%), plant height (30%) and youngest leaf expansion respond negatively to the flooding in sorghum as these traits have a direct influence on the photosynthetic rate under anaerobic conditions (Promkhambut et al. 2010). Soil tillage, increasing plant density, usage of organic and mineral fertilizers, and improved varieties directly influence the crop establishment under waterlogged condition (Traore et al. 2020). Differential expression of aquaporin genes through transcriptome profiling of contrasting sorghum genotypes in nodal root tips and nodal root basal regions has revealed genetic variation (Kadam et al. 2017). Further, 145 non-redundant transcription factor genes of NAC family have been characterized and showed their differential expression patterns over time in response to multiple abiotic stress factors including flooding in sorghum (Kadier et al. 2017). LEA proteins, particularly LEA-2 was upregulated under flooding conditions. A total of 22 paralogs and 12 orthologs of LEA genes were identified and studied in the context of abiotic stress tolerance including flooding in sorghum. Such investigations provide new understandings regarding the formation of LEAs in sorghum (Nagaraju et al. 2019).

5.8 Aluminium Tolerance

Grain sorghum production is largely hindered in aluminium rich acidic soils as it is highly sensitive to aluminium (Al) toxicity. Plant vigor and yields are significantly reduced owing to the inhibition of water and mineral uptake by aluminium in acidic soils. Significant variation for Al-toxicity tolerance in a set of 80 genotypes for root length in five-day-old seedlings was recorded and the genotype MCSR T33 recorded the maximum tolerance (Ringo et al. 2010). This genotype recorded relatively higher net root growth in aluminium treatment against control. Sensitive genotypes are more susceptible to Al in a pH range of 3.9 and 4.2 compared to the tolerant genotypes (Tan et al. 1993). The locus *ALT SB* is reported to regulate the Al tolerance and the *ALTSB/SbMATE* gene was mapped to sorghum linkage group 3 (Magalhaes et al. 2007). Markers from this region have been deployed by breeders to introgress favorable *SbMATE* alleles in susceptible sorghum genotypes (Anami et al. 2015). Gene coding for aluminium-activated citrate transporter which is known to confer Al-tolerance, a member of the multidrug and toxic compound extrusion (MATE), was identified through map-based positional cloning (Magalhaes et al. 2007). Four simple sequence repeats (SSRs), one sequence tag site (STS) and three inter-simple sequence repeat (ISSR) markers showed significant association with Al-tolerance. Two markers on chromosome 3 positioned close to *AltSB*, a locus linked to Al-tolerance gene (*SbMATE*) indicating their association with Al-tolerance (Too et al. 2018).

5.9 Genetic Resources of Tolerance Genes -Glimpses on Classical Genetics and Traditional Breeding

The genomic resources are vital for fostering the genetic understanding and breeding of sorghum for abiotic stress tolerance. However, the paradigm shifted from its primary focus on improving yield potential to yield stability, input-use efficiency and redefined to understand the impact of genotype and environment interactions. This requires the creation and utilization of genetic resources. The significant role of CWRs as genetic resources for crop improvement was envisaged (Sasaki and Antonio 2009). The potential untapped genetic resources of sorghum especially the wild/tertiary gene pool valued for useful traits associated with abiotic stress tolerance need to be exploited. The exploitation of extremely valuable traits from gene pool envisages the knowledge on the genetic information on these wild relatives and genomic insights on the mechanisms of varied traits. The genetic barriers in gene transfer between wild and cultivated sorghum species are challenging. With the recent advances in next-generation sequencing (NGS) technologies, more genomic data will become available to researchers which could be of use for targeted trait modification for abiotic stress tolerance.

BTx406 was used as donor parent to convert the mini-core collection of sorghum conversion program (SCP) lines for early maturity (*Ma* genes) and plant height (*Dw* genes) and following which phenotypic selection of advanced F_2 progeny for photoperiod insensitivity and short height was done in the temperate environment through repeated backcrossing to the exotic parent (Stephens et al. 1967). Sixty per cent of sorghum association panel (SAP) lines were converted lines, however, the panel also contained photoperiod-insensitive landraces and historic breeding lines (Casa et al. 2008). So far seven genotypes viz., B35, E36-1, QL41, SC56, SDS 1948-3, 296B and SC283 have been used for identifying QTLs for the stay-green trait (Crasta et al. 1999; Haussmann et al. 2002; Sanchez et al. 2002; Harris et al. 2007; Srinivas et al. 2009; Habyarimana et al. 2010; Sabadin et al. 2012). Many studies have attempted to use the identified QTLs for stay-green leaf trait as well as drought tolerance for developing drought-tolerant cultivars through marker-assisted backcrossing (MABC) (Kassahun et al. 2010; Jordan et al. 2012; Vadez et al. 2013). A reference set of 96 genotypes was developed based on detailed multilocation drought response phenotyping of 258 diverse sorghum genotypes and their molecular characterization using 39 polymorphic SSR markers (Rakshit et al. 2016). Introgression of the stay-green QTLs suggested the role of *stg3A* and *stg3B* QTLs in transpiration efficiency and vapour pressure deficit (Vadez et al. 2011). B35 donor parent alleles at stay-green QTL *stg1* contributed to increased water extraction differently in moderately senescent caudatum genotype, and highly senescent durra genotype (Vadez et al. 2011). Better donors for each of the component traits of abiotic stress tolerance, which depends on the genetic backgrounds, environment regimes need to be pinned down and used in the breeding programs. Sorghum genotypes exhibit significant differences for salt tolerance despite its moderate tolerance levels (Swami et al. 2011). Salt stress in sweet sorghum increased the germination duration (Gill et al. 2003), while decreased the germination percentage (Almodares et al. 2007). Wide variation among sorghum cultivars was noticed for the sensitivity of germination to high salinity (Samadani et al. 1994). The accumulation of toxic ions (Na^+ and Cl^-) causes disturbances in ion uptake and K^+ status of tissues; thus, it is the high K^+/Na^+ discrimination and the low Na^+/K^+ ratio maintenance in tissues determine the salt-tolerant genotypes (Amtmann et al. 1999). Jambo, a salt-tolerant sorghum variety in comparison with salt-sensitive genotypes, has shown to accumulate fewer Na^+ and maintained lower Na^+/K^+ ratios in the root and shoot tissues (Bavei et al. 2011). Preferential deposition of Na^+ ions in the shoot at the leaf base was noted (de Lacerda et al. 2003); an increased level of Ca^{2+} in the culture solution enhanced growth but decreased sodium uptake of sorghum plants (Asghar et al. 2009). The control of the excess accumulation of ROS generated as secondary stress under high salinity is also an essential component of salt tolerance. Application of silicon to soil alleviated salinity stress in two sorghum cultivars along with enhanced antioxidant activity and activity of antioxidant enzymes viz., ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), peroxidase (PRO), glutathione reductase (GR) (Kafi et al. 2011). Among these cultivars, the cv. Keller registered the highest stem yield and sucrose content at higher salinity level (Almodares et al. 2008).

Three high yielding mutant varieties with early maturity, semi-dwarf stature (PAHAT, SAMURAI 1 and SAMURAI 2) as well as drought-tolerance suitable for the dry season drought-prone areas of Indonesia were developed. Further, some sorghum mutant lines with acid soil tolerance have been identified (Human 2020).

5.10 Genomic Resources

5.10.1 Brief Account of Molecular Mapping of Tolerance Genes and QTLs

Molecular genetics and genomics could accelerate plant breeding through DNA marker-based selection by the way of saving time and resources (Foolad, 2007; Collard and Mackill, 2008). Genetic dissection of genomic regions governing critically important traits can be achieved through the QTLomic approach if robust QTLs have been mapped (Collard et al. 2005; Mohammadi-Nejad et al. 2008; Jena et al. 2010). Genes/QTLs responsible for abiotic stress tolerance have been identified through conventional breeding and QTL analysis in sorghum (Collins et al. 2008; Takeda and Matsuoka 2008). QTL mapping continues to remain relevant in plants given the complex nature of the genome and interaction among multiple genes for any given economically important trait (Korte and Farlow 2013).

Nested Association Mapping (NAM) populations are suitable for mapping QTLs for complex traits and such an effort in sorghum is relevant to study genes relevant to climate change (Yu et al. 2008). Advances in statistical tools coupled with high performing computer in mapping approaches of NAM populations help to detect small effect loci with minimal false-positives (Bouchet et al. 2017). Multi-parent advanced generation intercross populations (MAGIC) can aid in overcoming the limitation of traditional linkage analysis with RILs and NAMs (Boyles et al. 2019). A MAGIC population in sorghum created through random mating consisting of 1000 inbred accessions derived from 19 diverse founder lines is considered as a valuable resource (Ongom and Ejeta 2018).

Novel approaches of forward and reverse genetics have great potentiality in determining the genetic basis of traits to contribute to breeding more climate-resilient crops including sorghum. The original sorghum reference genotype ‘BTx623’ was subjected to ethyl methane sulphonate (EMS) and a set of two populations were developed and characterized by sequencing: sequence information of 486 lines (PRJNA297450) serves as a reference for deeper analysis of mutations in specific genes (Xin et al. 2008). A Targeting Induced Local Lesions IN Genomes (TILLING) population of 1600 lines has also been generated through EMS mutagenesis in sorghum genotype BTx623 and its applicability has been evaluated on a subset of mutant lines (Xin et al. 2008). Additional resources for sorghum include mutant populations that are either being screened for target traits such as epicuticular wax or being developed as TILLING populations (Xin et al. 2008).

5.10.2 Genomics-Aided Breeding for Tolerance Traits

The availability of the sorghum genome sequences and global gene expression studies using NGS and high-throughput gene expression platforms (McCormick et al. 2018) could uncover gene networks that contribute to the abiotic stress tolerance of sorghum. The NGS and advanced metabolic profiling might impact the field of QTLomics and would facilitate the cloning of important genes regulating abiotic stress tolerance. Resequencing of several accessions or RILs in combination with statistical linkage analysis could pave way for marker-assisted mapping (Zheng et al. 2011). Gene expression and function need to be assessed under drought stress and several experimental techniques are being employed including functional genomic platforms (Rhee and Mutwil 2014). Finding the relative expression of a particular gene in particular stress or combined stress will help us to decode the role of the genes (Zheng et al. 2011; Sanjari et al. 2019). The most inclusive transcriptomic data for sorghum are accessible through Phytozome (McCormick et al. 2018). 47 RNA-sequencing (RNAseq) profiles from various time-points across major plant organs of the sorghum 'BTx623' reference genome were constructed and expression data was used to determine gene functionality. Although not as robust, the new 'Rio' reference genome also has expression data from various organs across developmental time-points, but not enough to be considered an expression atlas under different stress conditions (Dugas et al. 2011; Gelli et al. 2014; Chopra et al. 2015). As more sequencing is conducted, the data needs to be centralized for the access by a larger community.

Besides sequence-based information, adaptive responses of sorghum have been monitored by genome-wide expression analysis under different stress conditions such as salinity, osmotic stress, or abscisic acid (Chinnusamy et al. 2004). The members of the AP2/ERF transcription factor superfamily have been identified in sorghum (Yan et al. 2013). The chloroplast glutathione reductase (cpGRs), G-protein complexes, DREB proteins, and *Sorghum bicolor* expressed sequence tags (SbEST) have been reported to play very important roles in abiotic stress responses not only in sorghum but in other plant species as well. In a recent study, a total of 1910 DEGs under cold were identified between cold-tolerant genotype HongkeZi and cold-sensitive genotype BTx623 (Chopra et al. 2015). They could identify upregulation of TFs including DREB, C-repeat binding factors, and ERF TFs under cold stress in tolerant genotype HongkeZi. Various in silico genome-wide analyses of genes, promoters, or microRNAs (miRNAs) are being performed that will help in the identification and characterization of existing and new orthologs of these sequences. Transcript profiling following sodium chloride (NaCl) drought stress showed upregulation of the RGA1(I), but downregulation under higher temperature. An integration of abscisic acid, ethylene, auxin, and methyl jasmonate signalling was probably involved in regulating the expression of the drought response through the DREB transcription factors (Sanjari et al. 2019).

RNA-Seq technology in combination with the sorghum genome sequence has proved the involvement of *SbPIN4, 5, 8, 9,* and *11* (Paterson et al. 2009; Shen et al. 2010). Sorghum metabolic pathways database, identified over 50 differentially expressed drought-responsive gene orthologs with enriched ABREs and CGTCA-motifs or motifs responsive to ABA specific to sorghum (Buchanan et al. 2005; Dugas et al. 2011). The global transcriptome of leaf and root tissues could elucidate temporal patterns in response to drought stress and hold in characterising QTLs/genes involved in the stay-green phenotype (Varoquaux et al. 2019). Four sorghum genotypes were subjected to polyethene glycol (PEG)-induced drought stress at the seedling stage to profile the transcriptome in response to stress. 180 genes were found to be differentially regulated in response to stress (Abdel-Ghany et al. 2020).

5.11 Recent Concepts and Strategies

The genomics of sorghum has been studied extensively and multiple throughput datasets are publicly available. By utilizing the bioinformatics platform, identification of putative candidate genes involved in stress response has been attempted and further used in the genotyping of the breeding populations for the identification of superior lines (Shinozaki et al. 2018). Homozygous sorghum genotype BTx623 of 730 Mb genome was sequenced and analyzed to trace the similarity, repetitive elements, gene models and miRNAs. The heterochromatin region of sorghum was high (460 Mb) compared to rice (63 Mb). Retrotransposons in sorghum were revealed to be 55% which is between the maize genome (79%) and the rice genome (26%). Transposon coverage was 7.5% which was intermediate between maize (2.7%) and rice (13.7%), and a homology-based and ab initio gene prediction methods identified over 27,640 protein-coding genes (Paterson et al. 2009). Reference genome order and coverage were improvised by the development of high-density genetic map using 10,000 markers for genotyping RIP derived from BTx623 and IS3620C. Mapping could lead to the integration of seven additional contigs spanning 24.64 Mbp on to the reference genome (McCormick et al. 2018). Resequencing of inbred lines, parents and genotype panels has led to the identification of many events across the genome and their evolutionary implications in sorghum. Further, these genomic data sets have set a platform to accelerate the breeding for abiotic stress tolerance in sorghum.

Nanotechnology is finding increasing relevance in medicine, agriculture and engineering fields. Crop plants interact with engineered nanomaterials (ENMs) as well as unintentional emissions of nanoparticles originating from natural or nanotechnologies in agriculture and food sectors. The exposure of nanoparticles is expected to have a concern in agriculture and environmental landscapes besides human health (Kranjc and Drobne 2019). A range of field problems including biotic

stresses, abiotic stresses, nutrient use efficiency, nutrient deficiency etc., are being addressed using various nanoparticles. The effects of nanoscale zinc oxide (ZnO-NP), calcium oxide (CaO-NP) and magnesium oxide (MgO-NP) on growth and productivity of sorghum have been documented (Naseeruddin et al. 2018). Nutrient uptake was significantly better with the foliar application compared to other methods of application. ZnO-NP amendment recorded increased grain yield and Zn-enrichment has also been achieved: these findings suggested a nanotechnology-based strategy for enhancing crop productivity and grain nutritional quality (Dimkpa et al. 2017). Nanoparticles have a significant influence on enhanced drought stress tolerance and increased flux of proteins involved in oxidation-reduction, ROS detoxification, stress signalling, and hormonal pathways (Hasanuzzaman et al. 2020). Application of Si-NP increased yield and yield-related attributes besides imparting drought tolerance in sorghum (Ahmed et al. 2011).

Several miRNAs associated with gene expression regulation of plant stress responses as well as in the biosynthesis pathways of carbon, glucose, starch, fatty acid, and lignin and xylem formation, that have a key role in abiotic stress tolerance in sorghum have been identified. These have the potentiality in the development of next-generation designer sorghum (Rajwanshi et al. 2014). The imposition of moisture stress in sorghum genotype IS1945 resulted in upregulation of several differentially expressed miRNAs involved in the regulation of transcription (Pasini et al. 2014). Upregulation of sorghum genes homologous with rice miRNA 169 g during drought stress condition evidenced the role of the miRNA in drought stress tolerance (Zhao et al. 2007). Involvement of miRNAs in water stress response in sorghum was also proved through *in silico* analysis of *cis*-elements of miRNA targets like genes for transcription factors, chaperonins and metabolic enzymes (Ram and Sharma 2013). Hence, miRNA 169 g has been considered as a potential candidate target for designing drought-tolerant sorghum genotypes through genetic engineering. Further, long noncoding RNAs (*lncRNAs*) from foxtail millet, having sequence conservation and collinearity with sorghum responded to simulated drought stress (Qi et al. 2013).

Targeted gene modification allows the ability to eliminate deleterious genetic variants in regions that are tightly linked to beneficial alleles, creating linkage drag. Rather it is difficult to handle through the conventional backcross breeding technique. Functional validation and crop improvement for abiotic stress tolerance through genetic transformation/genome editing is a way forward in genomic designing for abiotic stress in sorghum. However, the deployment of CRISPR will still depend on public perception.

Genomic selection has become a method of choice or advanced breeding program for quantitative and complex traits (Kulwal 2016). Use of genomic selection in sorghum is still in its nascent stage. The application of genomic prediction models for plant height showed a significantly high correlation between predicted plant height and observed plant height measurements (Watanabe et al. 2017). Further, moderate to high predictability for grain yield was reported in sorghum (Hunt et al. 2018). More recently, genomic prediction for grain yield and its components showed promise for mining useful sources of genetic variation

(Sapkota et al. 2020). Higher prediction accuracies in the range of 0.67–0.83 for biomass yield and related traits were obtained using genomic selection models (Yu et al. 2016). Further, there is a need to employ high-throughput trait-assisted genomic selection along with phenomics technologies to increase its predictability in diverse sorghum accessions (Fernandez et al. 2017) (Fig. 5.2).

High-throughput field phenotyping platforms and processing methods are currently being developed for several sorghum traits, including tillering, grain number and multiple leaf morphological characteristics. Accurate imaging and genetic algorithms are relevant to plant canopy modulation and manipulation (Thapa et al. 2018). Below-ground biomass traits are now possible to measure in a non-destructive way even in controlled conditions. Instrumentation and methodology to dissect root growth and morphology have been developed (Pineros et al. 2016; Joshi et al. 2017). High-throughput technologies with the development and testing of instrumentation for field phenotyping is gaining momentum. Ground robots (Fernandez et al. 2017), remote sensing (Shafian et al. 2018), advanced imaging (Potgieter et al. 2017), UAVs (Han et al. 2018) and machine learning (Vijayarangan et al. 2018) are the latest tools being employed as part of field-plot techniques in sorghum. High-throughput phenotyping platforms have been employed for successful phenotyping of stalk width (Gomez et al. 2018) and plant height (Fernandez et al. 2017; Watanabe et al. 2017; Hu et al. 2018; Pugh et al. 2018) in sorghum.

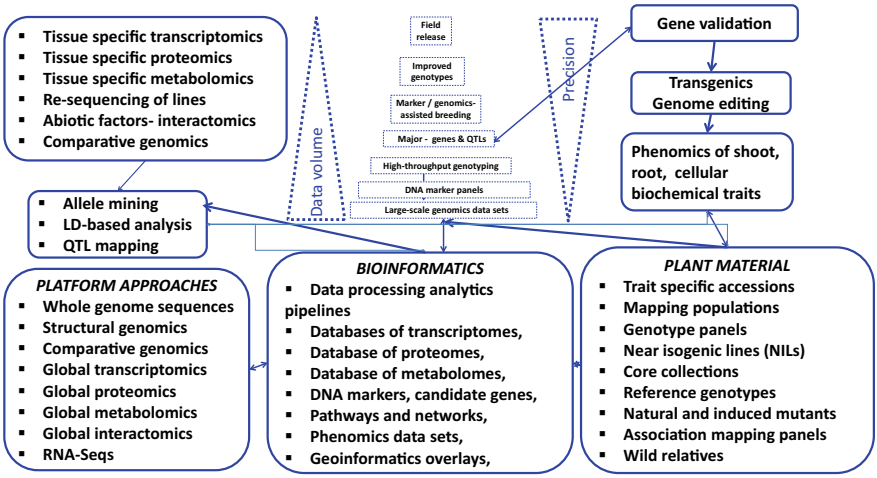

Fig. 5.2 An overview of the platform technologies and their application in sorghum research and breeding

5.12 Summary

The pressure from growing world population under unpredictable environments, losing farmlands and natural resources, necessitates reviving of the world's agricultural systems. Agriculture must adopt disruptive technologies to meet the needs of the global population. Latest climate projections based on previous trends are predicted to have negative impacts on agricultural crop productivity in future decades (Rosenzweig et al. 2014). Abiotic stresses limit the growth and productivity to varying degrees depending on the time of onset, duration, and intensity of stress and significant reductions in potential yield levels of sorghum was reported due to the detrimental effects of environmental stresses. Sorghum with its stay-green trait, C_4 photosynthesis, deep rooting system, high epicuticular wax and better WUE represents one of the model systems for trait oriented research. Considerable natural variation among different sorghum genotypes is reported which has great relevancy in sorghum improvement for target and stressed environments. A large number of genetic and genomic resources can provide the foundation to identify and understand the genes underlying phenotypic diversity and understanding G × E in sorghum. Insights into the physiological and molecular mechanisms of cold, salt, and aluminium tolerance besides drought tolerance along with the genetic and genomic resources paved the opportunities for researchers to relate sequence variations with phenotypic traits. Transcriptional modulations result in differential expression of genes functioning in multiple metabolic pathways of metabolic and physiological responses. With recent advances in next-generation sequencing technologies and high-throughput phenotyping platforms as well as fine mapping using advanced mapping populations such as NAM, backcross-derived NAM, and MAGIC populations have shown great promise in this research. With the sequencing of the sorghum genome, functional genomics has great scope in elucidating the roles of structural and regulatory genes involved in complex biological processes associated with abiotic stress responses (Paterson et al. 2009). The current resources and ensuing discoveries could enhance sorghum production along with its end-use quality in varying and contrasting environments to enhance global food security. Future functional genomic studies with the genes not yet annotated, but expressed only in the drought-resistant genotypes are expected to help in developing drought-resistant crops.

References

Abdel-Ghany SE, Ullah F, Ben-Hur A, Reddy AS (2020) Transcriptome analysis of drought-resistant and drought-sensitive sorghum (*Sorghum bicolor*) genotypes in response to peg-induced drought stress. IntL J Mol Sci 21:772

Ahmed M, Qadeer U, Aslam MA (2011) Silicon application and drought tolerance mechanism of sorghum. Afr J Agric Res 6:594–607

Akinseye FM, Ajeigbe HA, Traore PC, Agele SO, Zemadim B, Whitbread A (2020) Improving sorghum productivity under changing climatic conditions: a modelling approach. Field Crops Res 246:107685

Akman H, Zhang C, Ejeta G (2020) Physio-morphological, biochemical, and anatomical traits of drought-tolerant and susceptible sorghum cultivars under pre-and post-anthesis drought. Physiol Plant. https://doi.org/10.1111/ppl.13242

Almodares A, Hadi M, Ahmadpour H (2008) Sorghum stem yield and soluble carbohydrates under different salinity levels. Afr J Biotechnol 7(22):4051–4055

Almodares A, Hadi M, Dosti B (2007) Effects of salt stress on germination percentage and seedling growth in sweet sorghum cultivars. J Biol Sci 7:1492–1495

Amtmann A, Jelitto TC, Sanders D (1999) K+-selective inward-rectifying channels and apoplastic pH in barley roots. Plant Physiol 120:331–338

Anami SE, Zhang LM, Xia Y, Zhang YM, Liu ZQ, Jing HC (2015) Sweet sorghum ideotypes: genetic improvement of stress tolerance. Food Energy Secur 4(1):3–24

Angarawai I, Ajeigbe H, Yeye M, Aba D (2017) Characterization of landrace sorghum lines for utilization in hybrid parent development. In: Proceedings of the genetic society of Nigeria (GSN) 41st annual conference, pp 437–441

Anzoua KG, Yamada T, Henry RJ (2011) Miscanthus. In: Kole C (Ed) Wild crop relatives: genomic and breeding resources. In: Industrial crops. Springer, Berlin, Heidelberg, pp 157–164

Asghar D, Khan A, Collins J (2009) Effects of salinity on growth, ionic relations and solute content of *Sorghum bicolor* L. Moench. J Plant Nutr 32:1219–1236

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev Plant Sci 24:23–58

Bavei V, Shiran B, Arzani A (2011) Evaluation of salinity tolerance in sorghum (*Sorghum bicolor* L.) using ion accumulation, proline and peroxidase criteria. Plant Growth Regul 64:275–285

Bayu W, Rethman N, Hammes P (2005) Growth and yield compensation in sorghum (*Sorghum bicolor* L. Moench) as a function of planting density and nitrogen fertilizer in semi-arid areas of northeastern Ethiopia. S Afr J Plant Soil 22:76–83

Bekele WA, Fiedler K, Shiringani A, Schnaubelt D, Windpassinger S, Uptmoor R, Friedt W, Snowdon RJ (2014) Unravelling the genetic complexity of sorghum seedling development under low-temperature conditions. Plant, Cell Environ 37(3):707–723

Blum A (1996) Crop responses to drought and the interpretation of adaptation. Plant Growth Regul 20:135

Blum A (2017) Osmotic adjustment is prime drought stress adaptive engine in support of plant production. Plant, Cell Environ 40:4–10

Bonnett GR, Henry RJ (2011) Saccharum. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, industrial crops. Springer-Verlag, Berlin Heidelberg, Germany, pp 165–177

Borrell AK, Mullet JE, George-Jaeggli B, van Oosterom EJ, Hammer GL, Klein PE, Jordan DR (2014) Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. J Exp Bot 65:6251–6263

Bouchet S, Olatoye MO, Marla SR, Perumal R, Tesso T, Yu J, Tuinstra M, Morris GP (2017) Increased power to dissect adaptive traits in global sorghum diversity using a nested association mapping population. Genetics 206:573–585

Boyles RE, Brenton ZW, Kresovich S (2019) Genetic and genomic resources of sorghum to connect genotype with phenotype in contrasting environments. Plant J 97:19–39

Bray EA (1993) Molecular responses to water deficit. Plant Physiol 103:1035

Buchanan CD, Lim S, Salzman RA, Kagiampakis I, Morishige DT, Weers BD, Klein RR, Pratt LH, Cordonnier-Pratt M-M, Klein PE (2005) *Sorghum bicolor*'s transcriptome response to dehydration, high salinity and ABA. Plant Mol Biol 58:699–720

Burow G, Burke JJ, Xin Z, Franks CD (2011) Genetic dissection of early-season cold tolerance in sorghum (*Sorghum bicolor* (L.) Moench). Mol Breed 28:391–402

Cao W, Sun C, Li X, Qiu J, Liu R (2017) Methane production enhancement from products of alkaline hydrogen peroxide pretreated sweet sorghum bagasse. RSC Adv 7:5701–5707

Casa AM, Pressoir G, Brown PJ, Mitchell SE, Rooney WL, Tuinstra MR, Franks CD, Kresovich S (2008) Community resources and strategies for association mapping in sorghum. Crop Sci 48 (1):30–40

Chaudhari GN, Fakrudin B (2017) Candidate gene prediction and expression profiling of near-isogenic lines (NILs) carrying stay-green QTLs in rabi sorghum. J Plant Biochem Biotechnol 26(1):64–72

Chiluwal A, Bheemanahalli R, Kanaganahalli V, Boyle D, Perumal R, Pokharel M, Oumarou H, Jagadish SK (2020) Deterioration of ovary plays a key role in heat stress-induced spikelet sterility in sorghum. Plant, Cell Environ 43:448–462

Chinnusamy V, Schumaker K, Zhu JK (2004) Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. J Exp Bot 55:225–236

Chopra R, Burow G, Burke JJ, Gladman N, Xin Z (2017) Genome-wide association analysis of seedling traits in diverse Sorghum germplasm under thermal stress. BMC Plant Biol 17:12

Chopra R, Burow G, Hayes C, Emendack Y, Xin Z, Burke J (2015) Transcriptome profiling and validation of gene based single nucleotide polymorphisms (SNPs) in sorghum genotypes with contrasting responses to cold stress. BMC Genom 16:1–11

Collard BC, Jahufer M, Brouwer J, Pang E (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169–196

Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans Roy Soc B 363:557–572

Collins NC, Tardieu F, Tuberosa R (2008) Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiol 147:469–486

Crasta O, Xu W, Rosenow D, Mullet J, Nguyen H (1999) Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Mol Gen Genet 262:579–588

Dayakar Rao B, Ratnavathi C, Karthikeyan K, Biswas P, Rao S, Vijay Kumar B, Seetharama N (2004) Sweet sorghum cane for bio-fuel production: a SWOT analysis in Indian context. National Research Centre for Sorghum, Rajendranagar, Hyderabad, India

de Lacerda CF, Cambraia J, Oliva MA, Ruiz HA, Prisco JTn (2003) Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environ Exp Bot 49:107–120

Devnarain N, Crampton BG, Chikwamba R, Becker JV, O'Kennedy MM (2016) Physiological responses of selected African sorghum landraces to progressive water stress and re-watering. S Afr J Bot 103:61–69

Dillon SL, Shapter FM, Henry RJ, Cordeiro G, Izquierdo L, Lee LS (2007) Domestication to crop improvement: genetic resources for *Sorghum* and *Saccharum* (Andropogoneae). Ann Bot 100:975–989

Dimkpa CO, White JC, Elmer WH, Gardea-Torresdey J (2017) Nanoparticle and ionic Zn promote nutrient loading of sorghum grain under low NPK fertilization. J Agric Food Chem 65:8552–8559

Djanaguiraman M, Nair R, Giraldo JP, Prasad PVV (2018) Cerium oxide nanoparticles decrease drought-induced oxidative damage in sorghum leading to higher photosynthesis and grain yield. ACS Omega 3:14406–14416

Djanaguiraman M, Prasad PV, Seppanen M (2010) Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol Biochem 48:999–1007

Dugas DV, Monaco MK, Olson A, Klein RR, Kumari S, Ware D, Klein PE (2011) Functional annotation of the transcriptome of *Sorghum bicolor* in response to osmotic stress and abscisic acid. BMC Genom 12:514

Emebiri LC (2013) QTL dissection of the loss of green color during post-anthesis grain maturation in two-rowed barley. Theor Appl Genet 126:1873–1884

Evans J, McCormick RF, Morishige D, Olson SN, Weers B, Hilley J, Klein P, Rooney W, Mullet J (2013) Extensive variation in the density and distribution of DNA polymorphism in sorghum genomes. PLoS ONE 8:e79192

Fakrudin B, Kavil S, Girma Y, Arun S, Dadakhalandar D, Gurusiddesh B, Patil A, Thudi M, Bhairappanavar S, Narayana Y (2013) Molecular mapping of genomic regions harbouring QTLs for root and yield traits in sorghum (*Sorghum bicolor* L. Moench). Physiol Mol Biol Plant 19:409–419

Fawusi M, Agboola A (1980) Soil moisture requirements for germination of sorghum, millet, tomato, and celosia. Agron J 72:353–357

Fernandez MGS, Bao Y, Tang L, Schnable PS (2017) A high-throughput, field-based phenotyping technology for tall biomass crops. Plant Physiol 174:2008–2022

Flowers TJ, Gaur PM, Gowda CL, Krishnamurthy L, Samineni S, Siddique KH, Turner NC, Vadez V, Varshney RK, Colmer TD (2010) Salt sensitivity in chickpea. Plant, Cell Environ 33:490–509

Foolad MR (2007) Genome mapping and molecular breeding of tomato. Intl J Plant Genom 64358

Fracasso A, Trindade LM, Amaducci S (2016) Drought stress tolerance strategies revealed by RNA-Seq in two sorghum genotypes with contrasting WUE. BMC Plant Biol 16:1–18

Franks T, Powell K, Choimes S, Marsh E, Iocco P, Sinclair B, Ford C, Van Heeswijck R (2006) Consequences of transferring three sorghum genes for secondary metabolite (cyanogenic glucoside) biosynthesis to grapevine hairy roots. Transgen Res 15:181–195

Garrity DP, Sullivan CY, Watts DG (1984) Changes in grain sorghum stomatal and photosynthetic response to moisture stress across growth stages 1. Crop Sci 24:441–446

Gelli M, Duo Y, Konda AR, Zhang C, Holding D, Dweikat I (2014) Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. BMC Genom 15:179

Gill PK, Sharma AD, Singh P, Bhullar SS (2003) Changes in germination, growth and soluble sugar contents of *Sorghum bicolor* (L.) Moench seeds under various abiotic stresses. Plant Growth Regul 40:157–162

Gomez FE, Carvalho G, Shi F, Muliana AH, Rooney WL (2018) High throughput phenotyping of morpho-anatomical stem properties using X-ray computed tomography in sorghum. Plant Methods 14:59

Habyarimana E, Lorenzoni C, Busconi M (2010) Search for new stay-green sources in *Sorghum bicolor* (L.) Moench. Maydica 55:187

Hadebe S, Modi A, Mabhaudhi T (2017) Drought tolerance and water use of cereal crops: a focus on sorghum as a food security crop in sub-Saharan Africa. J Agron Crop Sci 203:177–191

Hale MG, Orcutt DM (1987) The physiology of plants under stress. John Wiley & Sons, New York

Hall A, Blümmel M, Thorpe W, Bidinger F, Hash C (2004) Sorghum and pearl millet as food-feed-crops in India. Anim Nutr Feed Technol 4:1–15

Hall AE (2000) Crop responses to environment. CRC Press, Boca Raton, FL

Han Y, Song L, Liu S, Zou N, Li Y, Qin Y, Li X, Pan C (2018) Simultaneous determination of 124 pesticide residues in Chinese liquor and liquor-making raw materials (sorghum and rice hull) by rapid multi-plug filtration cleanup and gas chromatography–tandem mass spectrometry. Food Chem 241:258–267

Harris K, Subudhi P, Borrell A, Jordan D, Rosenow D, Nguyen H, Klein P, Klein R, Mullet J (2007) Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. J Exp Bot 58:327–338

Harris-Shultz KR, Hayes CM, Knoll JE (2019) Mapping QTLs and identification of genes associated with drought resistance in sorghum. In: Sorghum. Humana Press, New York, NY, pp 11–40

Hasanuzzaman M, Bhuyan M, Parvin K, Bhuiyan TF, Anee TI, Nahar K, Hossen M, Zulfiqar F, Alam M, Fujita M (2020) Regulation of ROS metabolism in plants under environmental stress: a review of recent experimental evidence. Int J Mol Sci 21:8695

Haussmann B, Mahalakshmi V, Reddy B, Seetharama N, Hash C, Geiger H (2002) QTL mapping of stay-green in two sorghum recombinant inbred populations. Theor Appl Genet 106:133–142

House, LR (1985) A guide to sorghum breeding, 2nd edition. In: International crops research institute for the semi-arid tropics, Andhra Pradesh, India

Hsiao T, Fereres E, Acevedo E, Henderson D (1976) Water stress and dynamics of growth and yield of crop plants. In: Water and plant life. Springer, pp 281–305

Hu P, Chapman SC, Wang X, Potgieter A, Duan T, Jordan D, Guo Y, Zheng B (2018) Estimation of plant height using a high throughput phenotyping platform based on unmanned aerial vehicle and self-calibration: example for sorghum breeding. Eur J Agron 95:24–32

Human S (2020) Sorghum mutation breeding for Improving tolerance to abiotic stresses brought about by climate change. Joint FAO/IAEA programme-nuclear techniques in food and agriculture. http://www-naweb.iaea.org/nafa/news/pbg-sorghum-mutation.html

Hunt CH, van Eeuwijk FA, Mace ES, Hayes BJ, Jordan DR (2018) Development of genomic prediction in sorghum. Crop Sci 58:690–700

Jabereldar AA, El Naim AM, Abdalla AA, Dagash YM (2017) Effect of water stress on yield and water use efficiency of sorghum (*Sorghum bicolor* L. Moench) in semi-arid environment. IntL J Agric Forest 7:1–6

Jain V, Singla NK, Jain S, Gupta K (2010) Activities of enzymes of fermentation pathways in the leaves and roots of contrasting cultivars of sorghum (*Sorghum bicolor* L.) during flooding. Physiol Mol Biol Plant 16:241–247

Jena K, Kim S, Suh J, Kim Y (2010) Development of cold-tolerant breeding lines using QTL analysis in rice. In: Second Africa rice congress, Bamako, Mali, pp 22–26

Jordan DR, Hunt C, Cruickshank A, Borrell A, Henzell R (2012) The relationship between the stay-green trait and grain yield in elite sorghum hybrids grown in a range of environments. Crop Sci 52:1153–1161

Jordan WR (1980) Genetic variability in sorghum root systems: Implications for drought tolerance. In: Turner NC, Kramer PJ (eds) Adaptation of plants to water and high-temperature stress. John Wiley & Sons, New York, pp 383–399

Joshi DC, Singh V, Hunt C, Mace E, van Oosterom E, Sulman R, Jordan D, Hammer G (2017) Development of a phenotyping platform for high throughput screening of nodal root angle in sorghum. Plant Methods 13:56

Kadam S, Abril A, Dhanapal AP, Koester RP, Vermerris W, Jose S, Fritschi FB (2017) Characterization and regulation of aquaporin genes of sorghum [*Sorghum bicolor* (L.) Moench] in response to waterlogging stress. Front Plant Sci 8:862

Kadier Y, Zu Y-Y, Dai Q-M, Song G, Lin S-W, Sun Q-P, Pan J-B, Lu M (2017) Genome-wide identification, classification and expression analysis of NAC family of genes in sorghum [*Sorghum bicolor* (L.) Moench]. Plant Growth Regul 83:301–312

Kafi M, Nabati J, Masoumi A, Mehrgerdi MZ (2011) Effect of salinity and silicon application on oxidative damage of sorghum [*Sorghum bicolor* (L.) Moench.]. Pak J Bot 43:2457–2462

Kassahun B, Bidinger F, Hash C, Kuruvinashetti M (2010) Stay-green expression in early generation sorghum [*Sorghum bicolor* (L.) Moench] QTL introgression lines. Euphytica 172:351–362

Khan M, Jannat A, Munir F, Fatima N, Amir R (2020) Biochemical and molecular mechanisms of abiotic stress tolerance. In: Plant ecophysiology and adaptation under climate change: mechanisms and perspectives II. Springer, pp 187–230

Khatab I, El-Mouhamady A, Abdel-Rahman H, Farid MA, El-Demardash I (2017) Agro-morphological and molecular characterization of sorghum (*Sorghum vulgare* L.) for water stress tolerance. Intl J Curr Res Biosci Plant Biol 4:37–55

Khaton M, Sagar A, Tajkia J, Islam M, Mahmud M, Hossain A (2016) Effect of moisture stress on morphological and yield attributes of four sorghum varieties. Progress Agric 27:265–271

Kim JS (2003) Genomic analysis of sorghum by fluorescence in situ hybridization. PhD thesis, Texas A&M University, Texas

Kiranmayee K, Hash CT, Sivasubramani S, Ramu P, Amindala BP, Rathore A, Kishor P, Gupta R, Deshpande SP (2020) Fine-mapping of sorghum stay-green QTL on chromosome10 revealed genes associated with delayed senescence. Genes 11:1026

Knoll J, Ejeta G (2008) Marker-assisted selection for early-season cold tolerance in sorghum: QTL validation across populations and environments. Theor Appl Genet 116:541–553

Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:1–9

Kramer PJ (1983) Problems in water relations of plants and cells. Int Rev Cytol:253–286

Kranjc E, Drobne D (2019) Nanomaterials in plants: a review of hazard and applications in the agri-food sector. Nanomaterials 9:1094

Krupa K, Dalawai N, Shashidhar H, Harinikumar K (2017) Mechanisms of drought tolerance in Sorghum: a review. Intl J Pure Appl Biosci 5:221–237

Kulwal PL (2016) Association mapping and genomic selection—where does sorghum stand? In: Rakshit S, Wang Y-H (eds) The sorghum genome. Springer, Cham, Switzerland, pp 137–148

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

Leng B, Yuan F, Dong X, Wang J, Wang B (2018) Distribution pattern and salt excretion rate of salt glands in two recretohalophyte species of *Limonium* (Plumbaginaceae). S Afr J Bot 115:74–80

Mace E, Jordan DR (2011) Integrating sorghum whole genome sequence information with a compendium of sorghum QTL studies reveals uneven distribution of QTL and of gene-rich regions with significant implications for crop improvement. Theor Appl Genet 123:169

Mace ES, Tai S, Gilding EK, Li Y, Prentis PJ, Bian L, Campbell BC, Hu W, Innes DJ, Han X (2013) Whole-genome sequencing reveals untapped genetic potential in Africa's indigenous cereal crop sorghum. Nat Commun 4:1–9

Magalhaes JV, Liu J, Guimaraes CT, Lana UG, Alves VM, Wang Y-H, Schaffert RE, Hoekenga OA, Pineros MA, Shaff JE (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat Genet 39:1156–1161

Marla SR, Shiva S, Welti R, Liu S, Burke JJ, Morris GP (2017) Comparative transcriptome and lipidome analyses reveal molecular chilling responses in chilling-tolerant sorghums. Plant Genome 10:1–16

Maulana F, Weerasooriya D, Tesso T (2017) Sorghum landrace collections from cooler regions of the world exhibit magnificent genetic differentiation and early season cold tolerance. Front Plant Sci 8:756

Mayaki W, Stone L, Teare I (1976) Irrigated and non-irrigated soybean, corn, and grain sorghum root systems 1. Agron J 68:532–534

McCormick RF, Truong SK, Sreedasyam A, Jenkins J, Shu S, Sims D, Kennedy M, Amirebrahimi M, Weers BD, McKinley B (2018) The *Sorghum bicolor* reference genome: improved assembly, gene annotations, a transcriptome atlas, and signatures of genome organization. Plant J 93:338–354

Mohammadi-Nejad G, Arzani A, Rezai AM, Singh RK, Gregorio GB (2008) Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the saltol QTL. Afr J Biotechnol 7:730–736

Mundia CW, Secchi S, Akamani K, Wang G (2019) A regional comparison of factors affecting global sorghum production: the case of North America, Asia and Africa's Sahel. Sustainability 11:2135

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nagaraju M, Kumar SA, Reddy PS, Kumar A, Rao DM, Kavi Kishor P (2019) Genome-scale identification, classification, and tissue specific expression analysis of late embryogenesis abundant (LEA) genes under abiotic stress conditions in *Sorghum bicolor* L. PLoS ONE 14: e0209980

Naseeruddin R, Sumathi V, Prasad TN, Sudhakar P, Chandrika V, Ravindra Reddy B (2018) Unprecedented synergistic effects of nanoscale nutrients on growth, productivity of sweet

sorghum [*Sorghum bicolor* (L.) moench], and nutrient biofortification. J Agric Food Chem 66:1075–1084

Newton RJ, Bhaskaran S, Puryear JD, Smith RH (1986) Physiological changes in cultured sorghum cells in response to induced water stress: II. Soluble carbohydrates and organic acids. Plant Physiol 81:626–629

Ogbaga CC, Stepien P, Dyson BC, Rattray NJ, Ellis DI, Goodacre R, Johnson GN (2016) Biochemical analyses of sorghum varieties reveal differential responses to drought. PLoS ONE 11:e0154423

Ongom PO, Ejeta G (2018) Mating design and genetic structure of a multi-parent advanced generation intercross (magic) population of sorghum (*Sorghum bicolor* (L.) Moench). Genes Genom Genet 8:331–341

Orchard P, Jessop R (1984) The response of sorghum and sunflower to short-term waterlogging. Plant Soil 81:119–132

Ortiz-Bobea A, Wang H, Carrillo CM, Ault TR (2019) Unpacking the climatic drivers of US agricultural yields. Environ Res Lett 14:064003

Osuna-Ortega J, Mendoza-Castillo M, Mendoza-Onofre L (2003) Sorghum cold tolerance, pollen production. Maydica 48:125–132

Pandey K, Lahiani MH, Hicks VK., Hudson MK, Green MJ, Khodakovskaya M (2018) Effects of carbon-based nanomaterials on seed germination, biomass accumulation and salt stress response of bioenergy crops. PLoS One 13(8):e0202274

Pang H, Qin G, Tan Z, Li Z, Wang Y, Cai Y (2011) Natural populations of lactic acid bacteria associated with silage fermentation as determined by phenotype, 16S ribosomal RNA and recA gene analysis. Syst Appl Microbiol 34:235–241

Pardales J Jr, Kono Y, Yamauchi A (1991) Response of the different root system components of sorghum to incidence of waterlogging. Environ Exp Bot 31:107–115

Pasini L, Bergonti M, Fracasso A, Marocco A, Amaducci S (2014) Microarray analysis of differentially expressed mRNAs and miRNAs in young leaves of sorghum under dry-down conditions. J Plant Physiol 171:537–548

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A (2009) The *Sorghum bicolor* genome and the diversification of grasses. Nature 457:551–556

Petsakos A, Prager SD, Gonzalez CE, Gama AC, Sulser TB, Gbegbelegbe S, Kikulwe EM, Hareau G (2019) Understanding the consequences of changes in the production frontiers for roots, tubers and bananas. Glob Food Sec 20:180–188

Pineros MA, Larson BG, Shaff JE, Schneider DJ, Falcão AX, Yuan L, Clark RT, Craft EJ, Davis TW, Pradier PL (2016) Evolving technologies for growing, imaging and analyzing 3D root system architecture of crop plants. J Integr Plant Biol 58:230–241

Pontieri P, Del Giudice F, Dimitrov MD, Pesheva MG, Venkov PV, Di Maro A, Pacifico S, Gadgil P, Herald TJ, Tuinstra MR (2016) Measurement of biological antioxidant activity of seven food-grade sorghum hybrids grown in a Mediterranean environment. Aust J Crop Sci 10:904

Potgieter AB, George-Jaeggli B, Chapman SC, Laws K, Suárez Cadavid LA, Wixted J, Watson J, Eldridge M, Jordan DR, Hammer GL (2017) Multi-spectral imaging from an unmanned aerial vehicle enables the assessment of seasonal leaf area dynamics of sorghum breeding lines. Front Plant Sci 8:1532

Prasad P, Djanaguiraman M, Perumal R, Ciampitti IA (2015) Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and thresholds for temperature and duration. Front Plant Sci 6:820

Prasad PV, Pisipati S, Mutava R, Tuinstra M (2008) Sensitivity of grain sorghum to high temperature stress during reproductive development. Crop Sci 48:1911–1917

Promkhambut A, Polthanee A, Akkasaeng C, Younger A (2011) A flood-free period combined with early planting is required to sustain yield of pre-rice sweet sorghum (*Sorghum bicolor* L. Moench). Acta Agric Scand B Soil Plant Sci 61:345–355

Promkhambut A, Younger A, Polthanee A, Akkasaeng C (2010) Morphological and physiological responses of sorghum (*Sorghum bicolor* L. Moench) to waterlogging. Asian J Plant Sci 9:183

Pugh NA, Horne DW, Murray SC, Carvalho G Jr, Malambo L, Jung J, Chang A, Maeda M, Popescu S, Chu T (2018) Temporal estimates of crop growth in sorghum and maize breeding enabled by unmanned aerial systems. Plant Phenome J 1:1–10

Qi X, Xie S, Liu Y, Yi F, Yu J (2013) Genome-wide annotation of genes and noncoding RNAs of foxtail millet in response to simulated drought stress by deep sequencing. Plant Mol Biol 83:459–473

Rajabi Dehnavi A, Zahedi M, Ludwiczak A, Cardenas Perez S, Piernik A (2020) Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. Agronomy 10:859

Rajwanshi R, Chakraborty S, Jayanandi K, Deb B, Lightfoot DA (2014) Orthologous plant microRNAs: microregulators with great potential for improving stress tolerance in plants. Theor Appl Genet 127:2525–2543

Rakshit S, Hariprasanna K, Gomashe S, Ganapathy K, Das I, Ramana O, Dhandapani A, Patil J (2014) Changes in area, yield gains, and yield stability of sorghum in major sorghum-producing countries, 1970 to 2009. Crop Sci 54:1571–1584

Rakshit S, Swapna M, Dalal M, Sushma G, Ganapathy K, Dhandapani A, Karthikeyan M, Talwar H (2016) Post-flowering drought stress response of post-rainy sorghum genotypes. Indian J Plant Physiol 21:8–14

Ram G, Sharma AD (2013) In silico analysis of putative miRNAs and their target genes in sorghum (*Sorghum bicolor*). Intl J Bioinformatics Res Appl 9:349–364

Rao BD, Patil J, Reddy KN, Soni VK, Srivatsava G (2014) Sorghum: an emerging cash crop. Foundation Books

Rhee SY, Mutwil M (2014) Towards revealing the functions of all genes in plants. Trends Plant Sci 19:212–221

Rhodes DH, Hoffmann L, Rooney WL, Herald TJ, Bean S, Boyles R, Brenton ZW, Kresovich S (2017) Genetic architecture of kernel composition in global sorghum germplasm. BMC Genom 18(1):1–8

Ringo J, Mneney E, Onkware A, Were B, Too E, Owuoche J, Gudu S (2010) Tolerance to aluminium toxicity in Tanzanian sorghum genotypes. Afr Crop Sci J 18:155–164

Rooney WL, Aydin S (1999) Genetic control of a photoperiod-sensitive response in *Sorghum bicolor* (L.) Moench. Crop Sci 39(2):397–400

Rosenzweig C, Elliott J, Deryng D, Ruane AC, Müller C, Arneth A, Boote KJ, Folberth C, Glotter M, Khabarov N, Neumann K (2014) Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. PNAS 111(9):3268–3273

Sabadin P, Malosetti M, Boer M, Tardin F, Santos F, Guimaraes C, Gomide R, Andrade C, Albuquerque P, Caniato F (2012) Studying the genetic basis of drought tolerance in sorghum by managed stress trials and adjustments for phenological and plant height differences. Theor Appl Genet 124:1389–1402

Samadani B, Kholdbarin B, Almodares A (1994) Salt tolerance of sweet sorghum cultivars and their mechanisms. M Sc thesis in biology (plant physiology). Shiraz University, Iran

Sanchez A, Subudhi P, Rosenow D, Nguyen H (2002) Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). Plant Mol Biol 48:713–726

Sanjari S, Shirzadian-Khorramabad R, Shobbar Z-S, Shahbazi M (2019) Systematic analysis of NAC transcription factors' gene family and identification of post-flowering drought stress-responsive members in sorghum. Plant Cell Rep 38:361–376

Sapkota S, Boyles R, Cooper E, Brenton Z, Myers M, Kresovich S (2020) Impact of sorghum racial structure and diversity on the genomic prediction of grain yield components. Crop Sci 60:132–148

Sasaki T, Antonio BA (2009) Sorghum in sequence. Nature 457:547–548

Schittenhelm S, Schroetter S (2014) Comparison of drought tolerance of maize, sweet sorghum and sorghum-sudangrass hybrids. J Agron Crop Sci 200:46–53

Seki M, Kamei A, Yamaguchi-Shinozaki K, Shinozaki K (2003) Molecular responses to drought, salinity and frost: common and different paths for plant protection. Curr Opin Biotechnol 14:194–199

Shafian S, Rajan N, Schnell R, Bagavathiannan M, Valasek J, Shi Y, Olsenholler J (2018) Unmanned aerial systems-based remote sensing for monitoring sorghum growth and development. PLoS ONE 13:e0196605

Shen C, Bai Y, Wang S, Zhang S, Wu Y, Chen M, Jiang D, Qi Y (2010) Expression profile of PIN, AUX/LAX and PGP auxin transporter gene families in *Sorghum bicolor* under phytohormone and abiotic stress. FEBS J 277:2954–2969

Shinozaki K, Yamaguchi-Shinozaki K (1996) Molecular responses to drought and cold stress. Curr Opin Biotechnol 7:161–167

Shinozaki Y, Nicolas P, Fernandez-Pozo N, Ma Q, Evanich DJ, Shi Y, Xu Y, Zheng Y, Snyder SI, Martin LB (2018) High-resolution spatiotemporal transcriptome mapping of tomato fruit development and ripening. Nat Commun 9:1–13

Singh P, Nedumaran S, Traore PCS, Boote KJ, Rattunde HFW, Prasad PV, Singh NP, Srinivas K, Bantilan MCS (2014) Quantifying potential benefits of drought and heat tolerance in rainy season sorghum for adapting to climate change. Agric For Meteorol 185:37–48

Singh V, Nguyen CT, van Oosterom EJ, Chapman SC, Jordan DR, Hammer GL (2015) Sorghum genotypes differ in high-temperature responses for seed set. Field Crops Res 171:32–40

Singla N, Jain V, Jain S, Sawhney S (2003) Activities of glycolytic enzymes in leaves and roots of contrasting cultivars of sorghum during flooding. Biol Plant 47:555–560

Srinivas G, Satish K, Madhusudhana R, Reddy RN, Mohan SM, Seetharama N (2009) Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. Theor Appl Genet 118:1439–1454

Stephens J, Miller F, Rosenow D (1967) Conversion of alien Sorghums to early combine genotypes. Crop Sci 7:396

Subudhi P, Rosenow D, Nguyen H (2000) Quantitative trait loci for the stay-green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. Theor Appl Genet 101:733–741

Sui N, Han G (2014) Salt-induced photoinhibition of PSII is alleviated in halophyte Thellungiella halophila by increases of unsaturated fatty acids in membrane lipids. Acta Physiol Plant 36:983–992

Sui N, Li M, Li K, Song J, Wang B-S (2010) Increase in unsaturated fatty acids in membrane lipids of *Suaeda salsa* L. enhances protection of photosystem II under high salinity. Photosynthetica 48:623–629

Sui N, Wang Y, Liu S, Yang Z, Wang F, Wan S (2018) Transcriptomic and physiological evidence for the relationship between unsaturated fatty acid and salt stress in peanut. Front Plant Sci 9:7

Swami AK, Alam SI, Sengupta N, Sarin R (2011) Differential proteomic analysis of salt stress response in *Sorghum bicolor* leaves. Environ Exp Bot 71:321–328

Takeda S, Matsuoka M (2008) Genetic approaches to crop improvement: responding to environmental and population changes. Nat Rev Genet 9:444–457

Tan K, Keltjens WG, Findenegg GR (1993) Aluminum toxicity in sorghum genotypes as influenced by solution acidity. Soil Sci Plant Nutr 39:291–298

Tao Y, Henzell R, Jordan D, Butler DR, Kelly A, McIntyre C (2000) Identification of genomic regions associated with stay green in sorghum by testing RILs in multiple environments. Theor Appl Genet 100:1225–1232

Taylor J, Duodu KG (2018) Sorghum and millets: chemistry, technology, and nutritional attributes, 2nd edn. Elsevier Inc

Thapa S, Zhu F, Walia H, Yu H, Ge Y (2018) A novel LiDAR-based instrument for high-throughput, 3D measurement of morphological traits in maize and sorghum. Sensors 18:1187

Thomas H, Howarth CJ (2000) Five ways to stay green. J Exp Bot 51:329–337

Too EJ, Onkware AO, Were BAi, Gudu S, Carlsson A, Geleta M (2018) Molecular markers associated with aluminium tolerance in *Sorghum bicolor*. Hereditas 155:20

Traore K, Traore B, Synnevåg G, Aune JB (2020) Intensification of sorghum production in flood recession agriculture in Yelimane, Western Mali. Agronomy 10:726

Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G (1997) Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Mol Breed 3:439–448

Upadhyaya HD, Wang Y-H, Sastry DV, Dwivedi SL, Prasad PV, Burrell AM, Klein RR, Morris GP, Klein PE (2016) Association mapping of germinability and seedling vigor in sorghum under controlled low-temperature conditions. Genome 59:137–145

Vadez V, Deshpande S, Kholova J, Ramu P, Hash CT (2013) Molecular breeding for stay-green: progress and challenges in sorghum. In: Varshney RK, Tuberosa, R (eds) Genomic applications for crop breeding, vol 2 (chap 8). Wiley & Son Publishers, New York, USA

Vadez V, Krishnamurthy L, Hash C, Upadhyaya H, Borrell A (2011) Yield, transpiration efficiency, and water-use variations and their interrelationships in the sorghum reference collection. Crop Pasture Sci 62:645–655

Varoquaux N, Cole B, Gao C, Pierroz G, Baker CR, Patel D, Madera M, Jeffers T, Hollingsworth J, Sievert J (2019) Transcriptomic analysis of field-droughted sorghum from seedling to maturity reveals biotic and metabolic responses. Proc Natl Acad Sci USA 116 (52):27124–27132

Venkateswaran K, Elangovan M, Sivaraj N (2019). Chapter 2: Origin, domestication and diffusion of *Sorghum bicolor*. In: Aruna C, Visarada KB, Bhat V, Tonapi VA (eds) Breeding sorghum for diverse end uses. Woodhead Publishing Series in Food Science, Technology and Nutrition, pp 15–31

Verma R, Kumar R, Nath A (2018) Drought resistance mechanism and adaptation to water stress in sorghum [*Sorghum bicolor* (L.) Moench]. Intl J Bio-resource Stress Manag 9:167–172

Vijayalakshmi K, Fritz AK, Paulsen GM, Bai G, Pandravada S, Gill BS (2010) Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Mol Breed 26:163–175

Vijayarangan S, Sodhi P, Kini P, Bourne J, Du S, Sun H, Poczos B, Apostolopoulos D, Wettergreen D (2018) High-throughput robotic phenotyping of energy sorghum crops. In: Hutter M, Siegwart R (eds) Field and service robotics. Springer proceedings in advanced robotics 5. Springer, pp 99–113. https://doi.org/10.1007/978-3-319-67361-5_7

Walulu RS, Rosenow DT, Wester DB, Nguyen HT (1994) Inheritance of the stay-green trait in sorghum. Crop Sci 34:970–972

Wang H, Wang R, Liu B, Yang Y, Qin L, Chen E, Zhang H, Guan Y (2020) QTL analysis of salt tolerance in *Sorghum bicolor* during whole-plant growth stages. Plant Breed 139:455–465

Watanabe K, Guo W, Arai K, Takanashi H, Kajiya-Kanegae H, Kobayashi M, Yano K, Tokunaga T, Fujiwara T, Tsutsumi N (2017) High-throughput phenotyping of sorghum plant height using an unmanned aerial vehicle and its application to genomic prediction modeling. Front Plant Sci 8:421

Wood AJ, Saneoka H, Rhodes D, Joly RJ, Goldsbrough PB (1996) Betaine aldehyde dehydrogenase in sorghum (molecular cloning and expression of two related genes). Plant Physiol 110:1301–1308

Xin Z, Wang ML, Barkley NA, Burow G, Franks C, Pederson G, Burke J (2008) Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. BMC Plant Biol 8:103

Xu W, Rosenow D, Nguyen H (2000) Stay green trait in grain sorghum: the relationship between visual rating and leaf chlorophyll concentration. Plant Breed 119:365–367

Yan K, Chen P, Shao H, Shao C, Zhao S, Brestic M (2013) Dissection of photosynthetic electron transport process in sweet sorghum under heat stress. PLoS ONE 8(5)

Yang Z, Li J-L, Liu L-N, Xie Q, Sui N (2020) Photosynthetic regulation under salt stress and salt-tolerance mechanism of sweet sorghum. Front Plant Sci 10:1722

Yu J, Holland JB, McMullen MD, Buckler ES (2008) Genetic design and statistical power of nested association mapping in maize. Genetics 178:539–551

Yu J, Tuinstra MR (2001) Genetic analysis of seedling growth under cold temperature stress in grain sorghum. Crop Sci 41:1438–1443

Yu M, Li J, Chang S, Zhang L, Mao Y, Cui T, Yan Z, Luo C, Li S (2016) Bioethanol production using the sodium hydroxide pretreated sweet sorghum bagasse without washing. Fuel 175: 20–25

Zhang D-f, Zeng T-r, Liu X-y, Gao C-x, Li Y-x, Li C-h, Song Y-c, Shi Y-s, Wang T-y, Yu L (2019) Transcriptomic profiling of sorghum leaves and roots responsive to drought stress at the seedling stage. J Integr Agric 18:1980–1995

Zhao B, Liang R, Ge L, Li W, Xiao H, Lin H, Ruan K, Jin Y (2007) Identification of drought-induced microRNAs in rice. Biochem Biophys Res Commun 354:585–590

Zheng L-Y, Guo X-S, He B, Sun L-J, Peng Y, Dong S-S, Liu T-F, Jiang S, Ramachandran S, Liu C-M (2011) Genome-wide patterns of genetic variation in sweet and grain sorghum (*Sorghum bicolor*L). Genome Biol 12:R114

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 6:66–71

Chapter 6
Genomic Designing for Abiotic Stress Tolerance in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]

C. Tara Satyavathi, Supriya Ambawat, Deepmala Sehgal, Charu Lata, Shalini Tiwari, Rakesh K. Srivastava, Sudhir Kumar, and Viswanathan Chinnusamy

Abstract Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the fifth most important cereal crop followed by rice, wheat, maize, sorghum and is well-adapted to survive under drought, high temperature, salinity, lodging and poor soils. It is cultivated on 29 million ha in the arid and semi-arid tropical regions of Asia and Africa and is used as a staple food for around 90 million people. Pearl millet being a climate-resilient crop is very important to mitigate the adverse effects of changing climate and can also ensure increased income and food security. Various abiotic stresses are major threat for its growth and development causing severe losses in its yield potential. Among these abiotic stresses, drought stress is the most devastating constraint that can occur at any growth stage in pearl millet causing yield losses of upto 55–67%. During the last several decades, there has been lot of progress in

C. Tara Satyavathi (✉) · S. Ambawat
ICAR-AICRP on Pearl Millet, Mandor, Agriculture University, Jodhpur 342304, Rajasthan, India
e-mail: csatyavathi@gmail.com

D. Sehgal
International Maize and Wheat Improvement Centre (CIMMYT), Mexico City, Mexico

C. Lata
CSIR-National Institute of Science Communication and Information Resources, 14 Satsang Vihar Marg, New Delhi 110067, India

S. Tiwari
CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001, India

R. K. Srivastava
International Crops Research Institute for Semi Arid Tropics, Patancheru 502324, Telangana, India

S. Kumar · V. Chinnusamy
ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi 110012, India

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pearl millet genetic improvement in India using both conventional as well as genomic approaches. Recently reported genome sequence information and several genomic studies for drought tolerance emphasize the need for exploiting its valuable attributes. Hence, there is a need to use modern genomic tools and genomic designing approaches to accelerate pearl millet improvement programs. Genomic approaches and genomic tools can definitely speed up gene innovation, trait mapping and can help in understanding of several complicated gene pathways and their interactions. Molecular approaches can be utilized to edit and design pearl millet genome in order to better identify different genes and biochemical pathways governing agronomically important characters like yield, salinity tolerance, drought and heat resistance, rancidity etc. In addition to bioinformatics and systems biology, different "omics" approaches like transcriptomics, proteomics and metabolomics can be useful for quantitative and qualitative analysis of gene expression allowing more precise use of marker assisted selection (MAS) and transgenic technologies. Keeping this in view, we tried to review the efforts made in pearl millet research towards genetic enhancement, improvement in inheritance and stability of the drought tolerance traits and use of newly developed genomic tools.

Keywords Pearl millet · Abiotic stress · Drought tolerance · Climate-resilience · Genomic designing

6.1 Introduction

Pearl millet, *Pennisetum glaucum* (L.) R. Br.] is a C_4 plant and belongs to the family Poaceae. It is the staple food for around 90 million people and is grown on 29 mha in the arid and semi-arid regions of Asia and Africa. Pearl millet is known for its adaptation and survival under drought, salinity, heat, lodging and poor soils. These features enhance its preference for cultivation in arid and semi-arid areas of the world. It is a multipurpose crop. The grain apart from human consumption is also used as feed for animals in dairy and poultry, alcohol industry, starch industry, processed food industry etc. while green fodder and dry stover are used for cattle (Basavaraj et al. 2010). Different bakery products, extruded and weaning food items are also being made using pearl millet.

Pearl millet is a rich source of energy, carbohydrates, fat, ash, dietary fibers, iron and zinc. It has higher α-amylase activity and fiber content (1.2 g/100 g) in comparison to other grains and rightly termed as '*nutricereal*'. It is gluten free and keeps its alkaline properties even after being cooked. It is a good source of vitamins like riboflavin, niacin, thiamine and several minerals (2.3 mg/100 g) such as iron, zinc, magnesium, copper, potassium, manganese and phosphorous. It has

higher fat content (5 mg/100 g) with better digestibility and is also rich in unsaturated fatty acids (75%). It contains higher amount of SDS (slowly digestible starch) and RS (resistant starch) which is responsible for low glycemic index (GI) and is the requirement of the food habits, transforming diets and food industry (Kumar et al. 2016; Satyavathi 2019).

Abiotic stresses are mainly responsible for yield losses occurring every year in pearl millet. Although, pearl millet performs better than major cereals like rice and wheat in semi-arid regions but changing soil and climatic conditions impose threat to pearl millet cultivation and affect its production. Change in climatic conditions badly affects pearl millet production through drought, heat and flooding sometimes leading to total crop failure . Despite the fact, that pearl millet, is usually believed to be well adapted to salinity, drought, extreme temperatures and nutrient stress, it is susceptible towards different abiotic stresses similar to other crops (Shivhare and Lata 2017).

In the past several decades, there has been lot of progress in pearl millet genetic improvement in India. Previously, increased production was achieved with controlled hybrids and conventional breeding methods of selection (Yadav and Rai 2013) but later various biotechnological and genomic approaches were used for further improvement in pearl millet (Ambawat et al. 2020). Conventional approaches are time consuming while modern biotechnological tools aid in accelerating breeding programs.

6.2 Abiotic Stresses in Pearl Millet

6.2.1 Drought

Drought is defined as a transient decrease in moisture availability in which the quantity of available water is significantly below optimum for a specific duration. In general, meteorological, hydrological or agricultural terms can be added with drought to explain it more specifically. For a particular crop, when there is not enough soil moisture to meet its requirement at a particular time it is termed as agricultural drought. Drought generally causes an economic loss in rain-fed agriculture and it is displayed as a scarcity or lack of rainfall. Pearl millet is the principal crop in semi-arid and arid conditions where major abiotic stress is drought or low moisture influencing productivity. Pearl millet is generally considered as drought-tolerant crop, even then it shows yield reduction of about 55–67% under adverse conditions (Bray et al. 2000; Krishnamurthy et al. 2011). Various studies on pearl millet revealed that drought affects growth, yield, osmotic adjustment, pigment, water relations, membrane integrity and photosynthetic action (Sankar et al. 2013, 2014; Ajithkumar and Panneerselvam 2014).

Drought stress leads to accumulation of osmoprotectants like proline that acts during plant growth and development. Proline, an anti-oxidative defense molecule, is an outstanding osmolyte and a signaling molecule (Hayat et al. 2012). Osmolytes help the plants by maintaining osmotic balance or cell turgor, prevents leakage of electrolytes by stabilizing the membranes and proteins like RUBISCO (Ribulose 1,5-bisphosphate carboxylase) (Hayat et al. 2012) and mitochondrial electron transport complex II (Hamilton and Heckathorn 2001), subsequently determining concentration of reactive oxygen species (ROS) in plants. Screening for drought, biochemical and molecular characterization performed in pearl millet until now rarely looked upon increased proline content and its association with drought tolerance. The increased sucrose level, total soluble sugars, amylase activity, reducing sugars and water deficit lead to decreased starch concentration in grains. Further, metabolic changes, ionic imbalance, inhibition or reduction in enzymatic activity, cell expansion inhibition, variations in solute concentration or combinations of these factors result in water shortfall in the plants. At low water potential, stomatal closure and a decrease in photosynthetic activity take place under drought stress. Mobilization of stored sugars is essential in plants to compensate for reducing assimilates to the grains.

The growth stages of the crop also influence the low water stress. To understand the pearl millet adaptation mechanism to drought, efforts have been made to understand pearl millet's response against moisture stress at various growth stages. As a result, research in pearl millet focused towards examining the various outcomes of drought during different growth stages (Sankar et al. 2013, 2014). During the germination stage or seedling emergence stage, water stress creates fatality in seedlings, resulting in poor crop stand and low yields of pearl millet (Fig. 6.1). However, the drought stress has a minimal consequence on pearl millet grain yield after the establishment of the seedling (Lahiri and Kumar 1966). Seedling germination is dependent on the availability of water, and it determines the severity of drought stress. Drought influences seedling growth in many ways at the seedling phase. The availability and amount of moisture influences leaf appearance in seedling. Early drought will prolong the seedling phase, influencing the development of secondary roots and leaves in the plants. Soil moisture at the coleoptile node induces the formation of secondary roots. Secondary root development and their relative rates vary among diverse germplasm under various drought regimes. It has been shown that leaf development and secondary root formation are affected by water stress. When different concentrations of polyethylene glycol (PEG) 6000 were used to induce moisture stress, it significantly influenced shoot and root length, germination percentage as well as shoot/root ratio.

The vegetative growth is one of the critical stages in pearl millet. A notable negative impact on the yield performance was observed during the shift from vegetative to reproductive stage. Profuse and asynchronized tillering helps in adaptation against drought stress during the vegetative growth. The main shoot's dominance reduced during the vegetative phase resulting in additional tillers due to water stress. Besides this, abscisic acid accumulation takes place, resulting in reduced apical dominance. Closing stomata also helps to maintain the turgor

Fig. 6.1 Drought as a major constraint affecting sowing and seedling stage in Pearl millet

pressure in the cell, hence this stomatal function change leads to an improvement in the survival period under limited water supplies. In pearl millet, the main shoot's flowering time is delayed due to drought stress at the vegetative stage, resulting into a possibility for escaping from the flowering period which is the most sensitive stage until favorable conditions are achieved. Pearl millet genotypes having early flowering will show lower but active basal tillers, high harvest index and low biomass.

The flowering and grain filling stages of pearl millet are also very sensitive to water stress, resulting in decreased grain yield and its components. A reduction in the panicle number and grain mass decreases the yield. If final stress occurs after flowering, number of grains per panicle is normally less affected. The shortening of the grain filling period results in low grain mass, mainly due to decreased grain growth rate. It is caused by a restriction on the supply of the current assimilate. Late-flowering genotypes also have a longer (Growth Stage1) GS1 period, i.e., the time between the emergence of seedlings and the initiation of panicles is extended in comparison to early genotypes. Hence, these genotypes are able to avoid drought stress during the most important phases of growth. Translocation of stored assimilates between grain and leaves is the major adaptation in pearl millet during terminal drought stress. However, pearl millet is regarded as the most drought and heat tolerant among all the cereals due to its adaptive evolution by natural selection.

Drought avoidance (DA) is yet another way to deal with drought stress. It is the potential of the plant to tolerate water shortage with the extent and time of low water potential. DA signifies the judicious use of water for morphological and physiological mechanisms. Closing stomata helps to save water for plants when they feel a water deficit in the soil. At the initial stages of development in pearl millet, the rapid development of the primary root enables water acquisition from a deeper root zone (Passot et al. 2016). Pearl millet can withstand drought by slowing overall water loss and is known to be drought resistant by developing new leaves or buds. Pearl millet farmers cultivate high tillering landraces which have capabilities

to develop secondary tillers when the main culm is exaggerated by drought under highly drought-prone environments. Small panicles and small individual grain sizes are the special traits for these high tillering landraces typically to reduce the impairment of grain filling under dry conditions, as the yield of grain in pearl millet is closely associated with the number of grains. Landraces in dry regions are a strong example of drought-tolerant breeding systems (Kusaka et al. 2005). Many of the landraces have genes for drought resistance present in these traditional landraces which may be used for improving yield potential as they have lower yield potential when compared to improved cultivars (Yadav et al. 2011).

Pearl millet is mostly cultivated as a rainfed crop in regions having very little rainfall. Improving drought tolerance is a major breeding objective in pearl millet. Breeding for improved adaptation to drought is difficult depending upon several uncertainties linked to drought adaptation mechanisms such as severity, timing, duration and extensive genotype x environmental interactions. Drought tolerance is characterized as plants' capability to grow and produce satisfactory yields under periodic soil water deficits with minimal water supply. It enables plants to flourish in water-limited conditions based on its potential to maintain high plant water status (Blum 2005). This process plays a crucial role in maintaining drought resistance for pearl millet, as resistant genotypes may undergo grain filling and mitigate the dramatic impact on yield. In addition, plants often sense environmental factors and cause growth changes to complete the life cycle, and this is referred to as 'drought escape.' The initiation of flowering is a complex character that is influenced by the complexities of both autonomous and environmental factors (Andres and Coupland 2012).

Drought is regulated by several genes, as drought tolerance is a polygenic trait greatly affected by the environment (Hu and Xiong 2014). The study of plant responses to environmental stress is complicated and challenging in traditional plant breeding programmes. To address the main problem of drought tolerance, it may be beneficial to explore key functional components or mechanisms of drought tolerance. Conventional methods have helped to achieve some success in improving drought tolerance in pearl millet, although molecular breeding as an additional tool in recent times improved drought tolerance with greater precision. The use of molecular tools is essential and helpful in dissecting complex quantitative traits like drought resistance into contributing traits, each regulated by genes or QTLs. Few recent studies have helped to clarify the genetic factors regulating the duration of the cycle and the change from the vegetative to the reproductive stage in pearl millet. Polymorphism was found in the gene PgMADS11 of the MADS-box gene family, which is associated with the difference in the flowering time in case of pearl millet (Mariac et al. 2011).

6.2.2 Heat

Pearl millet needs an ideal temperature of approximately 35°C for seed germination, coleoptile elongation, photosynthetic activity as this crop is suited to hot arid zones where the mid-day surface temperature could reach 45°C. Pearl millet could survive above 35°C, which could be detrimental to other cereal crops. Seed germination has also been observed to occur in pearl millet at 35–45°C but continues to decline after 45°C. Seedlings of pearl millet are mainly susceptible towards high temperature during first ten days of sowing. The elevated temperature of the pearl millet influences both the seedling and the reproductive stages. Germination rate and germination percentage are found to be reduced with an increase in temperature. After 45°C, these are gradually affected, and both are adversely affected at 50°C. Under regulated environmental conditions at constant exposure to 47°C, germination is completely stopped. The effect of high temperature may be minimized in presence of adequate water availability for transpiration so that the leaves remain cool during the development of the seedlings. Heat shock proteins play a vital role after plants have been exposed to elevated temperatures under environmental regulation.

In the changing climate scenario, with increasing temperatures, the development and study of high-temperature tolerant crops have become a high priority in research. Drastic declines in the yield of cereal crops due to climate change and elevated temperatures can substitute maize and sorghum with pearl millet in some semi-arid areas of Africa and Asia, leading to significant changes in cropping patterns and crop production areas.

The results of high-temperature stress during the reproductive cycle vary from crop to crop. High-temperature stress (>35°C) for one hour was observed to cause spikelet sterility in rice. Similarly, temperatures above 36°C are recorded for reducing viability of pollen in maize which can effect yield reduction. Pearl millet can tolerate high temperatures of up to 42°C during flowering. During summers, hybrids of pearl millet with maturity of 80–85 days can withstand high temperature of 42°C at flowering and can produce 4–5 tons grain per hectare and 8–10 tons dry stover per hectare under irrigated and well-managed conditions. Therefore, it is important to identify the heat tolerant sources during flowering period in order to improve the summer season hybrid breeding program. Many research organizations have made attempts to perform experiments on both controlled environmental and field conditions, based on target populations. Under controlled conditions, heat tolerance screening is performed in growth chambers that subject pearl millet to high-temperature stress, while for heat tolerance screening experiments in the field, staggered sowing has to be done so that different stages are subjected to different temperature regimes. Weather loggers are installed in an experimental area to monitor hourly air temperatures. Heat tolerant sources can be identified by observing and recording wide genetic variability for heat tolerance among pearl millet breeding lines and populations during reproductive stage.

Most pearl millet growth processes, such as seed germination, coleoptile elongation, photosynthesis etc. need an optimal temperature around 35°C, which is ideal for hot arid areas (Shivhare and Lata 2017). Although temperatures above 35°C may be detrimental to most other cereals' growth, pearl millet can thrive and maintain its optimum growth and yield potential even under hot environmental conditions (Yadav et al. 2010). Seed germination was observed to occur at 35–45°C in pearl millet and seedlings are most sensitive to high temperatures during the first ten days of sowing. Thus, to withstand high temperatures, the identification of genetic variations in pearl millet germplasm is essential. Pearl millet inbreed line H 77/833-2, commonly used in north western India, is tolerant for high temperature stress but vulnerable to terminal drought stress (Yadav et al. 2014).

6.2.3 Salinity

Salinity is an other important environmental constraint for crops in arid and semi-arid regions with high surface evaporation, low precipitation and poor irrigation patterns contributing to increased amounts of soluble salts that make groundwater unavailable to plants (Goyal 2004). It is estimated that more than 50% of the global cultivable land could face the extreme danger of salinization by 2050 (Chauhan et al. 2019). While pearl millet is a crop with a built-in ability to withstand soil salinity, it can therefore be grown for grain and forage production in saline soils. However, minimal information on pearl millet response to soil salinity is available to date. In water-limiting conditions with an increased occurrence of drought events combined with higher temperatures, salinization can rise. Flushing of salts using freshwater is expensive, and thus, the production of crops using salinity-tolerant crops is the only viable alternative. Pearl millet with natural resistance to salinity would be useful and can be used for grain and forage production in saline soils. Pearl millet variety 'HASHAKI 1' is a good forage variety and has been released in 2012 for saline areas like Uzbekistan. It may be used in breeding programs as salinity tolerant locally adapted cultivars (both OPVs and hybrids). Farmers of saline areas will be encouraged to adapt and expand pearl millet in the land which has not been cultivated for many years. Several parental lines and populations were identified in pearl millet possessing high grain yield ratio and stover yield ratio. A positive association was found between grain yield ratio and the stover yield ratio indicating that high stover yield ratio will be useful for genetic improvement in grain yield ratio. Simultaneous selection for both productivity and Salinity Tolerance Index (STI) will be extremely useful. Parental lines having differences in grain yield ratio have been identified to develop mapping population and identification of QTLs for salinity tolerance. Reduced shoot N content, increased Na^+ and K^+ content is generally correlated with salinity tolerance in pearl millet (Dwivedi et al. 2012). A related study also stated that under salinity, shoot biomass ratio and the Na^+ concentration could be considered as potential selection parameters during pearl millet germplasm screening (Krishnamurthy et al.

2007). In a salinity tolerance study performed by Raipuria (2012), pot experiments were conducted with 21 genotypes of pearl millet, in which ten seeds of each genotype were planted in pots 12 cm high and 8 cm diameter (12 × 8 cm). The pots were irrigated with varying NaCl solutions (50, 100, 150 and 200 mM) on alternate days. Observations were taken for shoot length, germination percentage, root length, root shoot length ratio, fresh and dry root and shoot weight, root shoot dry weight ratio and seedling vigor index (Fig. 6.2). Of the 21 genotypes studied, genotypes D23 and DPR18 exhibited salinity tolerance with good seedling vigor index and other seedling parameters tested (Fig. 6.3).

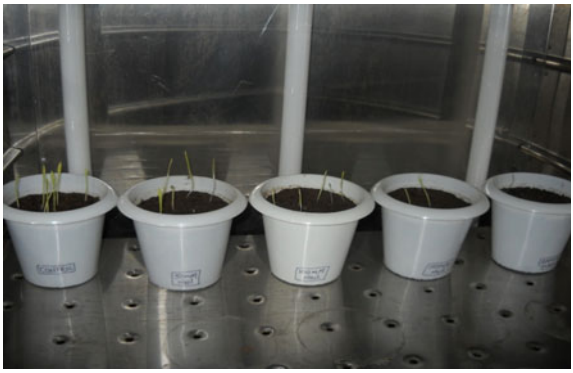

Fig. 6.2 Salinity stress tolerance studies in Pearl millet

Fig. 6.3 Performance of salinity tolerant pearl millet genotype DPR-18 seedlings at different concentrations of NaCl induced salinity under controlled conditions

6.3 Genetic Resources for Tolerance Genes

Based on genetic diversity studies of *Pennisetum* species three genepools were classified-primary, secondary and tertiary genepools (Harlan and De-Wet 1971). They were defined into cultivated *P. glaucum* based on their crossability, cross fertility and complexity of gene transfer. All types of weedy, cultivated and wild diploids ($2n = 2x = 14$) were included in primary gene pool while secondary gene pool comprised exclusively of tetraploid *P. purpureum* (Shum.) ($2n = 4x = 28$) and the tertiary gene pool contained widely related *Pennisetum* species of different ploidy levels (Dujardin and Hanna 1989).

Huge collection of germplasm of pearl millet exists at different national and international gene banks and detection and deployment of genetic variability in pearl millet for abiotic stress tolerance can help in enhancing its adaptation to various stresses. West Africa is considered to be the center of diversity for pearl millet (Senegal, Mali, Burkina Faso and Niger). A total of 1283 active pearl millet accessions have been collected at GRIN. Out of these, >75% were collected from India, Burkina, Nigeria, Faso and Zimbabwe. Genetic and genomic resources are very important for the improvement of crops as genetic resources provide primary source for breeding while genomic resources helps in identification of valuable genes, alleles and quantitative trait loci for crop improvement. Lot of genetic resources are available for pearl millet but this available information including physical/genetic maps and molecular markers is lesser in comparison to the other major cereals (Goron and Raizada 2015; Saha et al. 2016). These resources can be used further for genotyping, diversity analysis, physical and genetic-linkage mapping and QTL identification etc. Furthermore, improved knowledge of molecular and physiological mechanism along with precise phenotyping for abiotic stress tolerance can be highly useful for identification and utilization of new potential candidate genes to develop superior stress tolerance varieties.

Additionally, phenotypic screens have been developed at ICRISAT to classify tolerant germplasm for a variety of abiotic stresses (Krishnamurthy et al. 2007). Different sources and QTLs available in pearl millet germplasm for various abiotic stresses are described in Tables 6.1 and 6.2, respectively. Being a drought tolerant crop, extensive work was done in pearl millet at different developmental growth stages for understanding its response and adaptation mechanism under drought conditions. Water stress causes seedling death at germination or seedling stage leading to poor crop setting (Lata et al. 2015; Tiwari et al. 2016). Extreme drought at the seedling stage is the main reason of lower pearl millet production in the semi-arid regions (Shivhare and Lata 2017). An earlier report revealed that TNBH 0642, ICMV-221 and TNBH 0538 genotypes can perform better under PEG-induced drought stress at both early seedling and germination stages while PT6034 was found to be least resistant genotype (Govindaraj et al. 2010). It was observed that drought stress imposes less adverse affect on pearl millet grain yield after the seedling establishment (Lahiri and Kumar 1966). Due to asynchronous

tillering and rapid growth rate, drought stress has lesser or almost insignificant effect on yield and crop growth at vegetative stage and hence recovered very rapidly (Bidinger et al. 1987; Mahalakshmi et al. 1987). However, major significant reduction in pearl millet yield and stability is exerted by post-flowering or terminal drought stress (Mahalakshmi et al. 1987; Kholová and Vadez 2013).

6.4 Classical Genetics and Traditional Breeding

Research efforts for genetic improvement in pearl millet began in 1936 at Georgia Coastal Plain Experiment Station, Tifton, GA, US. This program was based on primary selection and genetic studies for development of superior cultivars sand inbred lines (Burton 1951). Pearl millet improvement has taken place in several phases. In the first phase, mode of pollination, flowering habit, cytogenetics, evaluation and enrichment of germplasm, cytoplasmic male sterility (CMS), genetic studies on agronomically important traits and detecting and using dwarf genes were focused by breeders. Pearl millet hybrid research has a significant impact and importance in India. In India, it was initiated in the 1940s by Indian Council of Agricultural Research (ICAR). Initial efforts were made during 1940s and 1950s for improvement of varieties for grain yield by mainly selecting local material (Singh et al. 2014). As a result, X1 and X2 were the two chance hybrids released in India for commercial utilization in fifties.

Hybrid development turned out to be the main target of breeders in 1960s for increasing the pearl millet production and productivity in India. HB-1' (Hybrid Bajra-1) released in 1965 was the first pearl millet hybrid (Athwal 1965). This hybrid increased the productivity and grain yield significantly in high temperature

Table 6.1 List of tolerant genotypes available for abiotic stress resistance in pearl millet

Abiotic stresses	Genotypes	References
Drought	CZP 9802; 863B, PRLT 2/89-33ICMP 83720, ICMV 9413, and ICMV 94472	Yadav et al. (2004), Dwivedi et al. (2012)
Heat	H77/833-2, H77/29-2, CVJ 2-5-3-1-3, 77/371 XBSECT CP1, 96AC-93, 1305, 77/371, Togo II, 99HS-18, G73107, 77/371	Sankar et al. (2013, 2014), Arya et al. (2014)
Salinity	ICMB 02111, ICMB 94555, ICMB 95333, ICMB 00888, ICMB 01222, ICMP 451, IP 3732, IP 3757, IP8210, and PRLT 2/89-33, 10876 and 10878 (Sudan), 18406 and 18570 (Namibia), and ICMV93753 and ICMV 94474 (India); 863-B, CZI 98-11, CZI 9621, HTP 94/54, DPR-18	Ali et al. (2004), Dwivedi et al. (2012), Raipuria (2012)

Table 6.2 Pearl millet QTLs associated with important traits under abiotic stress

QTL	Linkage group	Associated/Linked trait
Drought Tolerance-QTL (DT-QTL)	Linkage group-2	Terminal drought stress
Drought tolerance-QTL (DT-QTL)	Linkage group-2	Reduced salt uptake
Grain yield (GRYLD-QTL)	Linkage group-2	Drought tolerance in grain yield in early stress environments
Grain yield (GRYLD-QTL)	Linkage group-2, 3, 4 and 6	Drought tolerance in grain yield in late stress environments
Grain yield (GRYLD-QTL)	Linkage group-5	Drought tolerance in grain yield in early stress environments

Adapted from Shivhare and Lata (2017)

and drier regions of India. This was followed by a series of hybrids between 1965 and 1988 during the second phase. Till now, a total of 180 hybrids and 62 varieties have been identified and released for cultivation in different agro ecologies of India through ICAR-All India Coordinated Research Project on Pearl millet having different combinations of diverse phenotypic traits, offering many options to farmers of different ecologies (Satyavathi et al. 2020). Several recurrent selection methods, mass selection, S1 and S2 progeny selection, restricted recurrent phenotypic selection, half-sib selection, gridded-mass selection and full-sib selection were used in different population development programs with varying achievements in genetic improvement for different composites (Singh et al. 1988; Zaveri et al. 1989).

Later, during the third phase, marker-assisted selection (MAS) and marker-assisted backcrossing (MABC) were also included in pearl millet breeding program which proved quite useful for resistance breeding against downy mildew. During fourth phase, emphasis was laid on improving genetic diversity of pollinator parents and seed parents in order to improve abiotic stress tolerance and focusing towards specific niche areas (Govindaraj et al. 2010; Lata 2015) resulting into development and release of large number of cultivars and significant increase in the productivity (Kumara et al. 2014). Biofortification for micronutrients like zinc and iron in pearl millet grain (Rai et al. 2013; Kanatti et al. 2016) and application of molecular techniques were reinforced in the next phase to speed up cultivar development. Further, research was focused towards diversification of the restorer parents and seed, improvement in resistance against various diseases and development of extra-early hybrids for particular niche areas. Mutations induced by mutation breeding were also used for generating additional variability and identification of new alleles. Use of mutations was limited in pearl millet breeding due to availability of natural genetic variation and inadequate studies on chemical efficacy and rate along with physical mutagenesis impact on plant and seed characteristics (Acquaah 2007).

The genetic improvement program started and progressed effectively from selection of traditional and local genetic material to development of high-yielding,

disease resistant and abiotic stress tolerant hybrids. The foremost approach in hybrid breeding was based on strategic utilization of germplasm available in Indian subcontinent and Africa resulting into development of several diverse hybrids having different combinations of phenotypic traits which are very useful for adaptation in diverse ecologies. These cultivars were widely adopted by Indian farmers resulting in enhanced crop productivity from 305 kg ha^{-1} during 1951–1955 to 998 kg ha^{-1} during 2008–2012 and 1243 kg/ha during 2018–19 (Yadav and Rai 2013; Satyavathi et al. 2020).

Although conventional plant breeding and good agronomic practices have removed various constricts which decrease crop yield and effect nutritional quality, but there are several facets where the full potential of existing genetic resources have not been exploited completely. Support of genomics and genetic transformation technologies is required based on their deployment to deliver successful gene based techniques which can ultimately advance plant breeding programs for increasing abiotic stress tolerance in pearl millet contributing for advancement of sustainable agriculture in the dry areas. Vast development in the field of genomics, during past years has led to the availability and use of various novel tools for precise and faster breeding programs (Bollam et al. 2018; Meena et al. 2020; Srivastava et al. 2020). Huge marker data sets are used for genomics assisted breeding in pearl millet instead of one or a few loci linked with the trait.

6.5 Diversity Analysis

Characterization of the genomic diversity of crop germplasm is of vital importance for developing effective strategies for genetic improvement of agronomic traits. Extensive variation has been found in various agronomic traits in pearl millet germplasm such as days to flowering, panicle, grain and stover characteristics, grain nutritional composition and tolerance to various stresses (Bhattacharjee et al. 2007; Vadez et al. 2012; Amadou et al. 2013). Similarly, assessment of genetic diversity by molecular markers such as restriction fragment length polymorphism (RFLP; Bhattacharjee et al. 2002), amplified fragment length polymorphism (AFLP; Busso et al. 2000; Brocke et al. 2003) and simple sequence repeats (SSRs; Budak et al. 2003; Mariac et al. 2006; Oumar et al. 2008; Stitch et al. 2010; Nepolean et al. 2012; Bashir et al. 2014; Meena et al. 2020; Srivastava et al. 2020) markers has revealed tremendous diversity in both cultivated germplasm and landraces. However, most of these studies have been conducted with low density markers.

Advances in next generation sequencing (NGS) technologies have revolutionized the field of genomics (Metzker 2010). The reduced cost and time required to obtain information on several gigabases of nucleotide sequence have made NGS an attractive tool for genotyping large sets of accessions. As a result, thousands to millions of single nucleotide polymorphisms (SNPs) have been generated in almost

all crop species, even in species without a reference genome. Many NGS technologies have been deployed recently to unveil genetic diversity in many cereal crops (Onda and Mochida 2016). Genotyping by-sequencing (GBS), which allows simultaneous SNP discovery and genotyping, has been used extensively in many cereal crops to characterize germplasm (Fu and Peterson 2011; Poland and Rife 2012; Ramu et al. 2013; Sehgal et al. 2015; Moumouni et al. 2015). In pearl millet, 83,875 SNPs generated from GBS platform were utilized to assess the genetic diversity and population structure of 500 pearl millet accessions (Hu et al. 2015). These 500 accessions comprised of 248 landraces across Senegal and 252 accessions from Asia, Africa and America. The study revealed higher genetic diversity among accessions taken from Senegal in comparison to the other parts of the world (Hu et al. 2015). Recently, Kanfany et al. (2020) characterized 309 inbred lines, which were derived from African and Indian landraces and improved varieties, by 54,770 GBS-SNPs and reported higher nucleotide diversity in the panel as compared to the global collection analyzed in the study of Hu et al. (2015). By population structure analysis the authors detected five subgroups in the panel, which matched pedigree relationships of the inbred lines.

6.6 Association Mapping Studies

Association mapping (AM), also termed as linkage disequilibrium mapping, is an alternative approach for discovering QTLs, however, with much higher resolution compared to traditional QTL mapping. In AM, natural variation in germplasm collections is explored to identify marker-trait associations (Zhu et al. 2008). Since AM bypasses the need to develop mapping populations, it saves time and labor and enables the mapping of many traits in a single panel. Especially in the genomics era, where NGS tools and technologies have led to generation of millions of genome wide markers, genome wide association study (GWAS) has become a leading approach for trait dissection.

The use of GWAS approach to dissect complex traits was initially reported in pearl millet by Saïdou et al. (2009). The authors investigated natural allelic variation of the eight genes controlling flowering pathways in a set of 90 inbred lines for identification of genes related to crop adaptations to diverse climatic conditions. The candidate genes investigated were *FLORICAULA*, *CRY2*, *GI*, *Hd3a*, *Hd6*, *PHYA*, *PHYB* and *PHYC*. They reported significant associations of polymorphisms found in the *PHYC* gene with multiple traits including days to flowering, spike length, and stem diameter. The association found in *PHYC* gene in a second association panel was also validated which comprised of analysis in an independent set of 598 pearl millet individuals collected from Niger. Saïdou et al. (2014) investigated 100 Kb region around the *PHYC* gene and using the same panel of 90 inbred lines to categorize tightly linked best candidate markers. 75 markers distributed along this 100 Kb region were explored for association with various agronomic traits in the association study. Furthermore, signature of selection was

also assessed in an independent data which further reinforced the importance of *PHYC* in controlling phenotypic variation. Mariac et al. (2011) researched on MADS-box gene family, laying a crucial role in vegetative and flower development, on 21 pearl millet populations covering geographical diversity of West Africa. They reported that polymorphism in *PgMADS11* gene was linked with days to flowering and allele frequencies of the gene were closely associated with annual rainfall.

Scientists at ICRISAT developed an excellent genetic resource in 2013 to initiate association mapping of plethora of traits including agronomic traits under drought stress conditions. This panel known as Pearl Millet inbred Germplasm Association Panel (PMiGAP) was comprised of 346 lines representing global pearl millet diversity. Sehgal et al. (2015) utilized PMiGAP for the first time for fine mapping of a drought tolerance (DT) QTL (localized on linkage group 2) using candidate gene-based association mapping (AM) approach. The authors investigated population structure in PMiGAP with genome wide 37 SSR markers and unveiled six subpopulations in this panel. The six subpopulations were comprised of lines with similar traits and/or shared pedigree. For candidate gene-based association analysis, PMiGAP was genotyped with SNPs and conserved intron spanning primers (CISP) markers identified from 17 candidate genes mapped in DT-QTL interval and phenotyped for yield and yield-related traits under optimally irrigated and drought conditions. The study also identified many significant marker-trait associations using mixed linear model some of which were suggested to be used for marker assisted selection (MAS). Most importantly, SNP, an InDel (Insertion/Deletion) marker in putative acetyl CoA carboxylase gene and chlorophyll a/b binding protein gene, respectively, were suggested to be worth using for MAS in pearl millet. Later, Gemenet et al. (2015) conducted whole genome association analysis for the first time using PMiGAP to identify stable QTLs for agronomic traits under high and low phosphorous conditions. 285 DArT markers were used on 151 PMiGAP lines and two significant associations were reported. Marker PgPb11603 exhibited stable association with days to flowering while marker PgPb12954 was found to be associated with grain yield.

Debieu et al. (2018) performed GWAS with SNPs coming from GBS platform on a panel from West Africa to identify QTLs for biomass production and for stay-green trait in early drought stress conditions. They reported co-location of genes involved in the sirohaem and wax biosynthesis pathways with two important QTLs for biomass production and stay green traits. Hitherto, GWAS has not been exploited to dissect genes/QTL for heat or salinity tolerance in pearl millet. Using genome wide 3,117,056 SNPs and the phenotypic data generated for 20 agro-morphological traits in a panel of 288 testcross hybrids, Varshney et al. (2017) reported significant association of the markers on pseudo molecules Pg1 and Pg5 with grain number per panicle under early and late drought stress conditions.

6.7 QTL Mapping of Tolerance Genes

The development of first linkage map with RFLP markers laid the foundation for the first QTL mapping study on drought tolerance (Yadav et al. 1999, 2002). Using recombinant inbred line (RIL) mapping population based on the cross H 77/833-2 × PRLT 2/89-33, a major QTL was mapped for drought tolerance (DT-QTL) on LG 2 explaining 32% of the variation for grain yield. In addition, QTLs for 100-seed mass, harvest index, panicle harvest index and panicle number per m^2 were also observed with DT-QTL. PRLT 2/89-33 allele was associated with increased drought tolerance at this QTL interval. The effect of this DT-QTL was also validated in another cross ICMB 841 × 863B (Yadav et al. 2004; Bidinger et al. 2007) using two testers. It was observed that in the high tillering tester line H 77/833-2 (tester 1), DT-QTL exerted its effect on grain yield via maintaining biomass yield, while in tester 2 PPMI 301 the QTL increased panicle grain number and harvest index. Later, two different MABC programs (Serraj et al. 2005; Yadav et al. 2011) were also undertaken in which 30% improvement in introgression lines was recorded for combining ability in grain yield.

Sharma et al. (2014) investigated the effects of DT-QTL under salt stress conditions. The time course changes in Na^+ concentration and its compartmentalization in different plant parts of drought tolerant (PRLT 2/89-33) and drought sensitive (H 77/833-2) parents along with two QTL-NILs (ICMR 01029 and ICMR 01040) were studied. It was observed that the Na^+ concentration reached its maximum within 24 h after salinity imposition in roots of the sensitive parent, whereas it continued to increase with time and reached at its maximum at 120 h stage in the tolerant parent PRLT 2/89-33 and the two NILs (ICMR 01029 and ICMR 01004). It was also reported that Na^+ ions accumulated preferentially in the older leaves of the tolerant parent and NILs, whereas in the sensitive parent all main stem leaves showed significantly higher Na^+ concentration regardless of their age. This study showed that DT-QTL has pleiotropic effects on both drought and salt tolerance and therefore is an important target for breeding.

To dissect the physiological mechanism(s) underpinning DT-QTL, Kholová and Vadez (2013) undertook transpiration rate (TR) mapping using a fine mapping population segregating for DT-QTL. In this study, the QTL for TR is co-mapped in the same interval as DT-QTL on linkage group 2 suggesting that DT-QTL minimizes water loss during vegetative stage thush conserving soil moisture and hence contributes to terminal drought tolerance. To further enhance the physiological understanding of DT-QTL, Tharanya et al. (2018) used various phenotyping platforms (pot culture, LeasyScan, Lysimeter, Field) to measure traits related to plant water use and crop production traits in the same fine mapping population as used by Kholová and Vadez (2013). Four genomic regions were identified on linkage group 2 and co-mapping of water use and agronomic traits in DT-QTL interval. These results suggest that agronomic traits assessed in the field have tight linkages with physiological traits measured earlier in the development process.

6.8 Marker Assisted Breeding for Tolerance Traits

Successful marker-assisted breeding has been done for the DT-QTL i.e. terminal drought tolerance QTL identified in two biparental populations based on the crosses H 77/833-2 × PRLT 2/89-33 and ICMB 841 × 863B (Yadav et al. 2002, 2004). This DT-QTL was identified as the prime target for MAS in pearl millet because of its high and consistent estimated effects on grain yield and panicle harvest index (PNHI) in late drought stress environments and the absence of QTL x environment interaction (Bidinger et al. 2007). The tolerant allele at this QTL was transferred into the background of terminal two drought sensitive H77/833-2 and 841B lines by marker-assisted backcrossing resulting in the development of first MAS pearl millet hybrid HHB67 Improved in 2005. The introgression lines showed superior terminal drought tolerance (Serraj et al. 2005). Further, Bidinger et al. (2007) found that an increase in grain yield of 12 g m^2 was obtained across all environments in combination with gains in both individual grain mass and PNHI by selecting this QTL. For individual environments, the gains in grain yield were 16 and 13-16 g m^2 in the absence of stress and in the terminal stress environments, respectively.

6.9 Map-Based Cloning of Tolerance Genes

Various molecular tools have been developed for breeding during last 10–15 years and molecular marker maps, expressed sequence tag (EST) libraries (Senthilvel et al. 2008; Rajaram et al. 2013), bacterial artificial chromosomes library (BACs) (Allouis et al. 2001), subtractive cDNA library (James et al. 2015), stress responsive Suppression subtractive hybridization (SSH) library (Meena et al. 2020) are available now and numerous quantitative trait loci (QTLs) for several traits, including drought have been identified as mentioned in Table 6.2.

6.10 Genomics-Aided Breeding for Tolerance Traits

Genomic selection (GS) is an alternative to MAS in which genetic value of selection candidates is predicted based on the genomic estimated breeding value (GEBV) instead of QTL. GEBVs of candidates are calculated from high-density markers distributed throughout the genome using the model standardized in a training population. Since, GEBVs are based on all markers including both minor and major marker effects; GS captures genetic variation better than MAS for the particular trait under selection. GS has been extensively investigated in animal research and in plants success has been achieved in major crops (Spindel et al. 2015; Crossa et al. 2010, 2017; Srivastava et al. 2020).

The availability of whole genome sequence data in pearl millet has opened possibilities of conducting various genetic analyses which were not possible before

Table 6.3 Functional validation of *P. glaucum* genes linked with abiotic stress response

Name of *P. glaucum* gene	Homo-/ Heterologous host	Stress tolerance	References
VDAC	Yeast	Salinity	Desai et al. (2006)
Rab 7	Tobacco	Drought, salinity, cold, heat	Agarwal et al. (2008)
Hsc 70	*E. Coli*	Salinity, dehydration, temperature	Reddy et al. (2010)
LEA	*E. Coli*	Salinity, dehydration, temperature	Reddy et al. (2012)
DHN	*E. Coli*	Salinity	Singh et al. (2015)
NAC	*Arabidopsis thaliana*	Salinity	Shinde et al. (2020)
Apx, Dhn, Hsc70	Tobacco	Drought, salinity, temperature	Divya et al. (2019)
ASR3	*Arabidopsis thaliana*	Drought	Meena et al. (2020)
Hsp10	Tobacco	Drought, salinity, temperature	Kummari et al. (2020)

like development of GS models (Varshney et al. 2017; Srivastava et al. 2020). Considering the diverse environments used for pearl millet hybrids cultivation, breeders would need efficient genomic prediction models to predict variable marginal environments. Scientists at ICRISAT have initiated development and validation of various GS models for plethora of traits in pearl millet using PMiGAP for which whole-genome resequencing (WGRS) data is now available (Srivastava et al. 2020). Varshney et al. (2017) tested RR-BLUP model for genomic prediction using WGRS data to estimate grain yield in 4 environments (early stress, irrigated, late stress, and across environments) and it was observed that across environments exhibited high prediction accuracies of the model.

Liang et al. (2018) used two genotyping approaches, RAD-seq and tGBS, for evaluating inbred pearl millet lines from ICRISAT using genomic prediction models for a number of agronomic traits. It was observed that tGBS produced more 2x informative SNPs with marker allele frequency >0.05 than RAD-seq that produced 6x more sequencing data per sample. Further, the prediction accuracies obtained were in the range of 0.73-0.74 for 1000 grain weight, 0.87-0.80 for days to flowering, 0.48-051 for grain yield and 0.72-0.73 for plant height by using RR-BLUP model.

Jarquin et al. (2020) used conventional GBS (cGBS) and tunable GBS (tGBS) to investigate the genomic prediction models for grain yield. For cross-validation, three schemes (CV2, CV1 and CV0) were followed for predicting tested and untested genotypes in observed and unobserved locations. The phenotypic data

from 320 pearl millet hybrids and 37 inbred parents included grain yield measurements collected in replicated yield trials across four environments in India in 2015. Interestingly, the predictive ability of the model was improved across the three locations by including G × E interactions. The predictive ability was lower for CV1 in comparison to CV2 as predictive ability of the models in scheme CV1 depended on only genomic relationships among the testing and training sets. In CV0 scheme, predictive ability largely relied on the phenotypic correlations between environments and Hisar and Jamnagar proven the best predictions. Regarding the two platforms, it was found that the tGBS platform provided better accuracy of hybrid performance or same accuracy as cGBS.

6.11 Recent Concepts and Strategies Developed

Our understanding of the regulation of complex regulatory networks during plants stress response and tolerance have significantly improved due to advances in structural and functional genomic approaches such as sequencing, genome mapping, studying the expression and function of plant genomes using gene silencing, targeted induced local lesion in genome (TILLING) and overexpression. Advances in genomics, stress biology and bioinformatics could help us to develop stress tolerant crops. Various crop improvement platforms, such as genetic maps, NGS, GWAS, GBS, expression profiling, synteny studies, QTL mapping, candidate gene identification and genetic engineering technologies have been used to generate crop varieties with enhanced stress tolerance and yield (Tiwari and Lata 2019). Availability of huge datasets through different 'omics' technologies can be successfully utilized to identify and functionally characterize candidate genes to be used in genomics-aided breeding or transgenic technology for tolerance traits. As the genome of pearl millet has been sequenced, the challenge remains to characterize thousands of genes crucial for abiotic stress response and tolerance (Varshney et al. 2017). A crucial strategy to determine gene function is to knock-down or suppress the expression of a gene while overexpression in homo- or heterologous system (s) under the influence of a constitutive or stress-inducible promoter is an additional powerful tool for gene characterization. Recently genome editing has come up as a very important functional genomics tool. Significant works have already been initiated in these areas in pearl millet. Among the numerous crop improvement approaches, both gene editing and nanotechnology approaches are going to play crucial roles in pearl millet improvement. Impact of these approaches on pearl millet growth is briefly described below:

6.11.1 Gene Editing

Genome engineering or genome editing with targeted nucleases, is a modern technique for improving different crops that promises a substantial increase in yield in the near future. Several studies recently used targeted nucleases like clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR associated protein (Cas), zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) to successfully alter genes via adding or removing important or undesirable traits in plants for functional studies of genes and crop improvement programmes (Tiwari and Lata 2019). These approaches open up new opportunities for developing improved crop lines including pearl millet. Recently, Chanwala et al. (2020) performed genome wide analysis of WRKY transcription factors in pearl millet (*P. glaucum* L.) under drought and salinity and suggested that *PgWRKY*s can be used to increase crop productivity by using genome editing techniques to ensure future food security.

6.11.2 Nanotechnology

Apart from gene editing, nano-technological based approach for better crop growth and enhanced production has also gained momentum. Among various nano-scale applications, nanofertilizers play crucial role for sustainable agricultural practices. A study reported that the application of zinc nanofertilizer exert positive impact on production in *Pennisetum americanum* (Tarafdar et al. 2014). A significant improvement in root length, root area, shoot length, chlorophyll content, plant dry biomass and enzyme activities were observed that leads to enhanced production of millet. In pearl millet, the optimum concentration of silver nanoparticles (40 ppm) significantly increased the seed germination, seedling vigor index, shoot length, root length, biomass accumulation and also improved the biochemical profile (Khan et al. 2019). Application of gold nanoparticles, biosynthesized from *Cassia auriculata* leaf extract impose positive effects on seed germination and seedlings growth in pearl millet (Praveen et al. 2016). Beside these examples, nanofertilizers applications also reported to mitigate various biotic and abiotic stress conditions in pearl millet. Application of biogenic originated zinc oxide nanoparticles extracted from *Eclipta alba* reduce downy mildew incidence in pearl millet via inhibiting spore germination of *Sclerospora graminicola* zoospore (Nandhini et al. 2019). They primed the seeds and made foliar applications of zinc oxide nanoparticles and also reported high germination percentages and seedling vigor in pearl millet. In another investigation, Nandini et al. (2017) studied the extent of downy mildew control in *P. glaucum* by *Trichoderma*-mediated selenium nanoparticles and reported that activity of downy mildew is inversely proportional to the nanoparticles size. Similarly, chitosan nanoparticles (CNP) have been tested for their effectiveness against downy mildew disease in pearl millet via nitric oxide

generation (Siddaiah et al. 2018). In case of abiotic stress, seed priming of pearl millet seeds with silver nanoparticles (Ag-NPs) alleviate the adverse effect of salt stress and enhance the growth of *P. glaucum* plants. Silver nanoparticles substantially increased the K^+, decreased the Na^+ uptake and retained Na^+/K^+ in millet plants (Khan et al. 2020). Interestingly, alongside the application of nanofertlizers for growth enhancement in pearl millet, pearl millet itself is used as a source for green synthesis of nanoparticles. A recent study reported the biosynthesis of starch nanoparticles from pearl and proso millet starches and revealed significant increment in nutraceutical potentials of both these millets (Jhan et al. 2020).

6.12 Genetic Engineering for Abiotic Stress Tolerance Traits

Presently, crops withstand many abiotic stresses in their natural environment, including drought, salinity and temperature stress. As a result of various abiotic stresses, >50% decrement have been recorded in average yields of important cereal crops (Tiwari et al. 2017). Since pearl millet is also not an exception to abiotic stresses, it is very essential to detect and utilize genetic variants of pearl millet for stress tolerance that might help us in improving its adaptation against different stresses. Identification and characterization of the unknown DNA stretches helps us to understand the complex physiological and molecular mechanism behind tolerance of abiotic stress, that leads to the production of superior stress tolerance varieties. To determine gene function, functional characterization of several important genes related to abiotic stress responses in *P. glaucum* have been also mentioned in Table 6.3.

6.13 Role of Bioinformatics as a Tool

Advances in structural and functional genomic methods such as genome sequencing and mapping, examining the expression and function of the genome using gene silencing, TILLING and over expression have greatly enhanced our understanding of complex regulatory networks of stress response and tolerance in plants. Large datasets made accessible by various 'omics' technologies can be effectively used to classify and functionally characterize candidate genes for use in genomics-assisted breeding or transgenic technology for tolerance traits. In order to fast-track the crop improvement programs of pearl millet, the whole genome sequencing of its reference genotype, Tift $23D_2B_1$-P1-P5, was carried out by Varshney et al. in 2017. Additionally, 994 pearl millet genotypes were also re-sequenced that included 963 inbred lines and 31 wild accessions single plants for a better understanding of its population structure, genetic diversity, evolution and domestication of this

important crop. Whole genome shotgun (WGS) and Bacterial artificial chromosome (BAC) sequencing were used to assemble the pearl millet genome.

Several bioinformatics approaches came into account after the whole genome sequencing of the pearl millet. NGS approaches along with transcript profiling studies has been performed under various stresses to resolve the issues arising due to large genome of pearl millet. Genome wide investigation of WRKY transcription factors and Hsp70 gene family of pearl millet was performed under dehydration and salinity stress (Divya et al. 2019; Chanwala et al. 2020). Transcriptome analysis was also performed by Sun et al. (2020), to study the alteration in whole RNA transcripts of pearl millet during heat and drought stress. Pearl millet miRNAs and their targets in response to salinity were also discovered using small RNA sequencing (Shinde et al. 2020). A comparative transcriptome was analyzed at both vegetative and flowering stages of a terminal drought tolerant pearl millet genotype, PRLT2/89-33, under drought stress (Shivhare et al. 2020). Overall, bioinformatics approaches help us to understand the molecular basis involved in various mechanisms and their interconnection such as identification of genes, proteins and metabolites involved in different molecular and signaling network.

6.14 Social, Political and Regulatory Issues

Pearl millet production has decreased due to various abiotic stresses but in addition; several other factors like low harvest index, cultivation on poor soils with lesser or no inputs, seeds fertilizers, lack of genetic resources, use of simple and lesser effective techniques, stability and inheritance of desired traits, consistency and cost efficacy and availability of different test environments are some of the main constraints in the way of increased production of pearl millet. Drought is the most distressing constraint as it can happen at any growth stage in pearl millet including pre-flowering and post- flowering stage (Rai et al. 1999). The hybrids and varieties developed so far are targeted for A and B agroecological zones while most of breeding programs fail to deliver products for A_1 agroecological zone which is the major pearl millet growing region (Fig. 6.4). This zone has vast variation in microclimate (day and night temperature and humidity) and soil apart from rainfall which requires proper quantification but despite the breeding efforts there is narrow cultivar diversity in this drought prone ecology thus leaving very less cultivar choice to the farmers. Hence, there is a high need to give priority to enhance cultivar diversity in the A_1 zone in coming years and continue generating breeding lines for regions with <400 mm annual rainfall. Emphasis should be given to breed open pollinated varieties (OPVs) for arid region as it's very difficult to breed sustainable high yielding hybrids in these areas. This will also reduce seed cost and farmers can retain seed for few generations thus reducing cost of cultivation. Moreover, a strong R $\times$ R and B $\times$ B programme should be initiated in arid/hyper arid region taking centres located in the A_1 and A zone. A strong R line programme will deliver better hybrids for the zone in the coming time. The available genetic

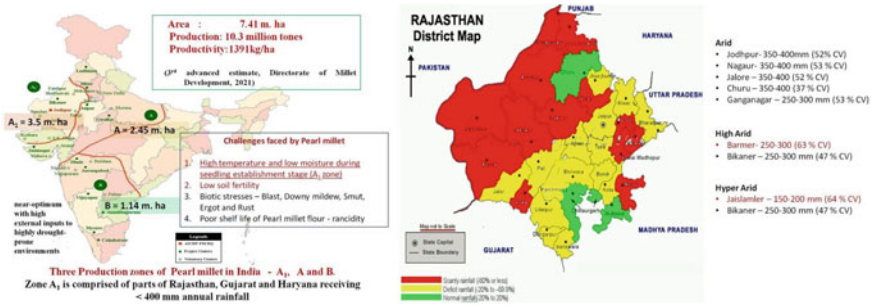

Fig. 6.4 Districts of Rajasthan (in A_1 zone) requiring drought tolerant pearl millet genotypes

stock of A/B pairs and R lines in the country should be scrupulously screened under arid and hyper arid regions to characterize and identify potential parents (Satyavathi et al. 2019). Developing adapted diversity for sustainable breeding progress, germplam conservation and pre-breeding, speed breeding, genetic and natural resource management, genomic tools, improved water conservation techniques, enhanced soil fertility and production practices are some of the other areas which can help in pearl millet crop improvement programs (Twomlow et al. 2008).

Various socio-economic constraints have also limited the pearl millet production and their consumption leading to a loss of cultivated diversity. Rural people of western Rajasthan prefer local landraces or 'Bajri' for their consumption. Hence, emphasis should be laid towards their development and use in the breeding programs. Landraces should be utilized to enrich the donor lines having alleles/genes lacking in the existing population to develop arid adopted base populations which will serve as mother stock for developing target lines of pearl millet breeding system in arid ecology. In addition, mechanized harvesting, development of pearl millet hybrids/varieties having enhanced regeneration ability and tolerant to salt/high temperature are some of the other areas which need to be focused to overcome the various issues related to the crop. Rancidity is another major issue associated with the pearl millet. Though, pearl millet has superior nutritional qualities but shelf life of its flour and bioavailability are major challenges in promotion of pearl millet products. Thus, focus should be put for enhancement of shelf life of pearl millet flour, biofortification for iron and zinc and producing reliable data on nutritional benefits and bioavailability of pearl millet. Varieties must be developed which possess capacity to enhance shelf life of flour along with reduced phenolic compounds and fat content. Moreover, there is a high need to explore its nutriceutical value and the different health benefits to make it popular among people. Drudgery is another social issue as pearl millet is mainly processed at household level in the rural areas using cumbersome methods involving a substantial drudgery which also needs attention.

Farmer participatory varietal improvement along with use of diverse gene pool can prove very useful in gaining genetic improvement in such situations. Such approaches can also meet out the needs of farmers for varietal adaptation against the changing and variable climatic conditions in a better way and speed up adoption by farmers (Christinck et al. 2005; Enjalbert et al. 2011). It can be further helpful and effective for deciding priority, identification of parental material, selection, testing and propagation. On the other hand, lack of availability of suitable market because of lesser consumer demand of pearl millet including farmers is an area of apprehension. Usually, this crop is being grown for food security instead of marketing which is a major obstacle for its growth and hence, the market participation and price is quite low in comparison to the superior cereals.

6.15 Future Perspectives

Pearl millet being a climate-resilient crop can prove to be a useful and excellent genomic resource for identification of candidate genes governing tolerance to different climatic and edaphic stresses. This can not only speed up its own genetic enhancement but can also be exploited for genetic improvement of other crops and can prove to be an alternative climate smart crop. Owing to its superiority for tolerance to drought, high temperature and salinity in comparison to other cereal crops, it has high prospective to turn out to be a substitute food and feed crop to ensure nutritional security. Huge collection of pearl millet germplasm and its characterization is important in order to develop a successful breeding program for pearl millet and to utilize them for targeting various traits and studying genetic variation. Further, collaborative attempts of breeders, physiologists, agronomists, policy makers and donors at both individual and institutional levels are highly needed to enhance the productivity of pearl millet under changing climatic conditions that causes various abiotic stresses affecting its productivity. Specific phenotyping platforms are required to assess high temperature and drought tolerance in order to combat various abiotic stresses and develop tolerant varieties.

Modern breeding tools and platforms along with genomic designing and genomic approaches are required to target important traits and hasten development of cultivars with the help of MAS or genomic selection. NGS techniques and high throughput genome analysis along with genome editing can prove very helpful for characterization of natural genetic variability existing in the pearl millet germplasm for abiotic stresses. Subsequently, discovery of sequence-based markers related to the major traits will be much useful in enhancing opportunities for pearl millet improvement, cultivar development in future. In addition, pearl millet has high nutritional value which can be exploited for improving nutritional quality and combating malnutrition. Hence, it can become an alternative crop for future. Speed breeding, a non-GMO path can be also much useful for the researchers in selecting plants for desired traits with numerous variations. Several advantages such as simplicity, high specificity and efficiency along with multiplexing associated with

emerging genomic tools and techniques over traditional methods make them highly effective and appreciable. Hence, it is concluded that use of genetic tools and resources along with speed breeding can facilitate plant biologists to extend their research in the field of crop improvement.

References

Acquaah G (2007) Principles of plant genetics and breeding, 2nd edn. Blackwell Publishing, Malden, MA, p 756. ISBN 978-0-470-66475-9

Agarwal PK, Agarwal P, Jain P, Jha B, Reddy MK, Sopory SK (2008) Constitutive overexpression of a stress-inducible small GTP-binding protein PgRab7 from *Pennisetum glaucum* enhances abiotic stress tolerance in transgenic tobacco. Plant Cell Rep 27:105–115. https://doi.org/10.1007/s00299-007-0446-0

Ajithkumar IP, Panneerselvam R (2014) ROS Scavenging system, osmotic maintenance, pigment and growth status of *Panicum sumatrense* Roth under drought stress. Cell Bio-chem Biophyss 68(3):587–595

Ali GM, Khan NM, Hazara R, Mcneilly T (2004) Variability in the response of pearl millet [*Pennisetum americanum* (L.) Leeke] accessions to salinity. Acta Agron Hung 52:277–286

Allouis S, Qi X, Lindup S, Gale MD, Devos KM (2001) Construction of a BAC library of pearl millet, *Pennisetum glaucum*. Theor Appl Genet 102(8):1200–1205

Amadou I, Gounga ME, Le GW (2013) Millets: nutritional composition, some health benefits and processing-a review. Emir J Food Agri 25:501–508

Ambawat S, Singh S, Shobhit, Meena RC, Satyavathi CT (2020) Biotechnological applications for improvement of the pearl millet crop. In: Gahlawat SK, Punia S, Siroha AK, Sandhu KS, Kaur M (eds) Pearl millet: properties, functionality and its applications, pp 115–138. Taylor & Francis (CRC Press), Boca Raton, Florida. https://doi.org/10.1201/9780429331732-7

Andres F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Nat Rev Genet 13:627–639

Arya R, Singh M, Yadav A, Kumar S (2014) Advances in pearl millet to mitigate adverse environment conditions emerged due to global warming. Forage Res 40:57–70

Athwal DS (1965) Hybrid bajara-1 marks a new era. Indian Farming 15(5):6–7

Basavaraj G, ParthasarathyRao P, Bhagavatula S, Ahmed W (2010) Availability and utilization of pearl millet in India. J SAT Agric Res 8:1–6

Bashir EMA, Ali AM, Ali AM, Mohamed ETI, Melchinger AE, Parzies HK et al (2014) Genetic diversity of Sudanese pearl millet (*Pennisetum glaucum* (L.) R. Br.) landraces as revealed by SSR markers, and relationship between genetic and agro-morphological diversity. Genet Resour Crop Evol 62:579–591

Bhattacharjee R, Bramel J, Hash T, Kolesnikova-Allen A, Khairwal S (2002) Assessment of genetic diversity within and between pearl millet landraces. Theor Appl Genet 105:666–673

Bhattacharjee R, Khairwal I, Bramel P, Reddy K (2007) Establishment of a pearl millet [*Pennisetum glaucum* (L.) R. Br.] core collection based on geographical distribution and quantitative traits. Euphytica 155:35–45

Bidinger FR, Mahalakshmi V, Rao GDP (1987) Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L) Leeke]. Factors affecting yields under stress. Aust JAgric Res 38:37–48. https://doi.org/10.1071/AR9870037

Bidinger FR, Nepolean T, Hash CT, Yadav RS, Howarth CJ (2007) Quantitative trait loci for grain yield in pearl millet under variable post flowering moisture conditions. Crop Sci 47:969–980

Blum A (2005) Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? Aust J Agric Res 56:1159–1168

Bollam S, Pujarula V, Srivastava RK, Gupta RK (2018) Genomic approaches to enhance stress tolerance for productivity improvements in pearl millet. In: Gosal SS, Wani SH (eds) Biotechnologies of crop improvement, vol 3. Springer. Berlin/Heidelberg, Germany, pp 239–264

Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. In: Gruissem W, Buchannan B, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, MD, pp 1158–1249

Brocke KV, Christinck A, Weltzien E, Presterl T, Geiger HH (2003) Farmer's seed systems and management practices determine pearl millet genetic diversity patterns in semiarid regions of India. Crop Sci 43:1680–1689

Budak H, Pedraza F, Cregan PB, Baenziger PS, Dweikat I (2003) Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. Crop Sci 43:2284

Burton GW (1951) Quantitative inheritance in pearl millet (*Pennisetum glaucum*). Agron J 43:409–417. https://doi.org/10.2134/agronj1951.00021962004300090001x

Busso CS, Devos KM, Ross G, Mortimore M, Adams WM, Ambrose MJ et al (2000) Genetic diversity within and among landraces of pearl millet (*Pennisetum glaucum*) under farmer management in West Africa. Genet Resour Crop Evol 47:561–568

Chanwala J, Satpati S, Dixit A, Parida A, Giri MK, 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):1–16

Chauhan PS, Lata C, Tiwari S, Chauhan AS, Mishra SK, Agrawal L, Chakrabarty D, Nautiyal CS (2019) Transcriptional alterations reveal *Bacillus amyloliquefaciens*-rice cooperation under salt stress. Sci Rep 9(1):1–13

Christinck A, Weltzien E, Hoffmann V (2005) Setting breeding objectives and developing seed systems with farmers: a handbook for practical use in participatory plant breeding projects. Margraf Publishers, Germany, p 188. ISBN 3-8236-1449-5

Crossa J, de Gustavo LC, Paulino P, Daniel G, Juan B, José LA et al (2010) Prediction of genetic values of quantitative traits in plant breeding using pedigree and molecular markers. Genetics 186:713–724. https://doi.org/10.1534/genetics.110.118521

Crossa J, Pérez-Rodríguez P, Cuevas J, Montesinos-López O, Jarquín D, De los Campos G et al (2017) Genomic selection in plant breeding: methods, models, and perspectives. Trends Plant Sci 22:961–975

Debieu M, Sine B, Passot S, Grondin A, Akata E, Gangashetty P et al (2018) Response to early drought stress and identification of QTLs controlling biomass production under drought in pearl millet. PLoS ONE 13(10): https://doi.org/10.1371/journal.pone.0201635

Desai MK, Mishra RN, Verma D, Nair S, Sopory SK, Reddy MK (2006) Structural and functional analysis of a salt stress inducible gene encoding voltage dependent anion channel (VDAC) from pearl millet (*Pennisetum glaucum*). Plant Physiol Biochem 44:483–493. https://doi.org/10.1016/j.plaphy.2006.08.008

Deshpande RS, Rao VM (2004) Coarse cereals in a drought-prone region: a study in Karnataka social and economic change, monograph Series No. 2. Institute for Social and Economic Change, Bangalore, India

Divya K, Kishor PK, Bhatnagar-Mathur P, Singam P, Sharma KK, Vadez V, Reddy PS (2019) Isolation and functional characterization of three abiotic stress-inducible (Apx, Dhn and Hsc70) promoters from pearl millet (*Pennisetum glaucum* L.). Mol Biol Rep 46(6):6039–6052

Dujardin M, Hanna WW (1989) Crossability of pearl millet with wild *Pennisetum* species. Crop Sci 29:77–80. https://doi.org/10.2135/cropsci1989.0011183X002900010019x

Dwivedi S, Upadhyaya H, Senthilvel S, Hash C (2012) Millets: genetic, and genomic resources. In: Janick J (ed) Plant breeding reviews, pp 247–375. John Wiley and Sons, Hoboken, NJ

Enjalbert J, Dawson JC, Paillard S, Rhoné B, Rousselle Y, Thomas M, Goldringer I (2011) Dynamic management of crop diversity: from an experimental approach to on-farm conservation. C R Biol 334(5–6):458–468. https://doi.org/10.1016/j.crvi.2011.03.005

Fu YB, Peterson GW (2011) Genetic diversity analysis with 454 pyrosequencing and genomic reduction confirmed the eastern and western division in the cultivated barley gene pool. Plant Genome 4:226–237

Gemenet DC, Hash CT, Sanogo MD, Sy O, Zangre RG, Leiser WL (2015) Phosphorus uptake and utilization efficiency in West African pearl millet inbred lines. Field Crops Res 171:54–66

Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a new green revolution. Front Plant Sci 6:157. https://doi.org/10.3389/fpls.2015.00157

Govindaraj M, Shanmugasundaram P, Sumathi P, Muthiah AR (2010) Simple, rapid and cost effective screening method for drought resistant breeding in pearl millet. Elec J Plant Breed 1:590–599

Goyal RK (2004) Sensitivity of evapotranspiration to global warming: a case study of arid zone of Rajasthan India. Agric Water Manag 69:1–11. https://doi.org/10.1016/j.agwat.2004.03.014

Hamilton EW, Heckathorn SA (2001) Mitochondrial adaptations to NaCl. Complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. Plant Physiol 126(3):1266–1274

Harlan JR, De-Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon 20:509–517. https://doi.org/10.2307/1218252

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments: a review. Plant Signal Behav 7:1–11

Hu H, Xiong L (2014) Genetic engineering and breeding of drought-resistant crops. Annu Rev Plant Biol 65:715–741

Hu Z, Mbacké B, Perumal R, Guèye MC, Sy O, Bouchet S, Prasad PVV, Morris GP (2015) Population genomics of pearl millet (*Pennisetum glaucum* (L.) R. Br.): comparative analysis of global accessions and Senegalese landraces. BMC Genomics 16(1):1048

James D, Tarafdar A, Biswas K, Satyavathi CT, Padaria JC, Kumar PA (2015) Development and characterization of a high temperature responsive subtractive cDNA library in Pearl millet (*Pennisetum glaucum* (L.) R. Br.). Indian J Exp Biol 53:543–550

Jarquin D, Howard R, Liang Z, Gupta SK, Schnable JC, Crossa J (2020) Enhancing hybrid prediction in pearl millet using genomic and/or multi-environment phenotypic information of inbreds. Front Genet 10:1294

Jhan F, Shah A, Gani A, Ahmad M, Noor N (2020) Nano-reduction of starch from under utilised millets: effect on structural, thermal, morphological and nutraceutical properties. Intl J Biol Macromol 159:1113–1121

Kanatti A, Rai KN, Radhika K, Govindaraj M (2016) Genetic architecture of open-pollinated varieties of pearl millet for grain iron and zinc densities. Indian J Genet 76:299–303. https://doi.org/10.5958/0975-6906.2016.00045.6

Kanfany G, Serba DD, Rhodes D, Bernardo A, Amand P, Gangashetty P et al (2020) Genomic diversity in pearl millet inbred lines derived from landraces and improved varieties. BMC Genome 21:469

Khan I, Raza MA, Awan SA, Khalid MHB, Raja N, Min S, Zhang A, Naeem M, Meraj TA, Iqbal N, Zhang, X (2019) in vitro effect of metallic silver nanoparticles (AgNPs): a novel approach toward the feasible production of biomass and natural antioxidants in pearl millet (*Pennisetum glaucum* L.). Appl Ecol Environ Res 17(6):12877–12892

Khan I, Raza MA, Awan SA, Shah GA, Rizwan M, Ali B, Tariq R, Hassan MJ, Alyemeni MN, Brestic M, Zhang X (2020) Amelioration of salt induced toxicity in pearl millet by seed priming with silver nanoparticles (AgNPs): the oxidative damage, antioxidant enzymes and ions uptake are major determinants of salt tolerant capacity. Plant Physiol Biochem 156:221–232

Kholová J, Vadez V (2013) Water extraction under terminal drought explains the genotypic differences in yield, not the anti-oxidant changes in leaves of pearl millet (*Pennisetum glaucum*). Funct Plant Biol 40:44-53

Krishnamurthy L, Serraj R, Rai KN, Hash CT, Dakheel AJ (2007) Identification of pearl millet [*Pennisetum glaucum* (L.) R. Br.] lines tolerant to soil salinity. Euphytica 158:179–188. https://doi.org/10.1007/s10681-007-9441-3

Krishnamurthy L, Zaman-Allah M, Purushothaman R, Irshad M, Vadez V (2011) Plant biomass productivity under abiotic stresses in SAT agriculture. In: Matovicb MD (ed) Biomass-detection, production and usage, pp 247–264. In Tech, London. https://doi.org/10.5772/17279

Kumar A, Metwal M, Kaur S, Gupta AK, Puranik S, Singh S et al (2016) Nutraceutical value of finger millet (*Eleusine coracana* (L.) Gaertn.), and their improvement using omics approaches. Front Plant Sci 7:934 https://doi.org/10.3389/fpls.2016.00934

Kumara CD, Bantilan C, Rajalaxmi A, Rai KN, Yadav OP, Gupta SK et al (2014) Development and diffusion of pearl millet improved cultivars in india: impact on growth and yield stability. ICRISAT, Patancheru, India

Kummari D, Bhatnagar-Mathur P, Sharma KK, Vadez V, Reddy PS (2020) Functional characterization of the promoter of pearl millet heat shock protein 10 (PgHsp10) in response to abiotic stresses in transgenic tobacco plants. Intl J Biol Macromol 156:103–110. https://doi.org/10.1016/j.ijbiomac.2020.04.069

Kusaka M, Ohta M, Fujimura T (2005) Contribution of inorganic components to osmotic adjustment and leaf folding for drought tolerance in pearl millet. Physiol Plant 125:474–489

Lahiri AN, Kumar V (1966) Studies on plant-water relationship III: further studies on the drought mediated alterations in the performance of bulrush millet. Proc Natl Inst Sci India B 32:116–129

Lata C (2015) Advances in omics for enhancing abiotic stress tolerance in millets. Proc Indian Natl Sci Acad 81:397–417

Lata C, Muthamilarasan M, Prasad M (2015) Drought stress responses and signal transduction in plants. In: Pandey GK (ed) The elucidation of abiotic stress signaling in plants, pp 195–225. Springer, New York, NY

Liang, Z, Gupta, SK, Yeh CT, Zhan, Y, Ngu DW, Kumar H et al (2018) Phenotypic data from inbred parents can improve genomic prediction in pearl millet hybrids. G3: Genes Genom Genet 8:2513–2522 https://doi.org/10.1534/g3.118.200242

Mahalakshmi V, Bidinger FR, Raju DS (1987) Effect of timing of water deficit on pearl millet (*Pennisetum americanum*). Field Crops Res 15:327–339. https://doi.org/10.1016/0378-4290(87)90020-7

Mariac C, Luong V, Kapran I, Mamadou A, Sagnard F, Deu M et al (2006) Diversity of wild and cultivated pearl millet accessions (Pennisetum glaucum [L.] R. Br.) in Niger assessed by microsatellite markers. Theor Appl Genet 114:49–58

Mariac C, Jehin L, Saïdou AA, Thuillet AC, Couderc M, Sire P (2011) Genetic basis of pearl millet adaptation along an environmental gradient investigated by a combination of genome scan and association mapping. Mol Ecol 20:80–91

Meena RP, Vishwakarma H, Ghosh G, Gaikwad K, Chellapilla TS, Singh MP, Padaria JC (2020) Novel ASR isolated from drought stress responsive SSH library in pearl millet confers multiple abiotic stress tolerance in PgASR3 transgenic Arabidopsis. Plant Physiol Biochem 156:7–19

Metzker ML (2010) Sequencing technologies—the next generation. Nat Rev Genet 11:31–46

Moumouni KH, Kountche BA, Jean M, Hash CT, Vigouroux Y, Haussmann BI, Belzile F (2015) Construction of a genetic map for pearl millet, *Pennisetum glaucum* (L.) R. Br., using a genotyping-by- sequencing (GBS) approach. Mol Breed 35(1):5–10

Nandhini M, Rajini SB, Udayashankar AC, Niranjana SR, Lund OS, Shetty HS, Prakash HS (2019) Biofabricated zinc oxide nanoparticles as an eco-friendly alternative for growth promotion and management of downy mildew of pearl millet. Crop Protec 121:103–112

Nandini B, Hariprasad P, Prakash HS, Shetty HS, Geetha N (2017) Trichogenic-selenium nanoparticles enhance disease suppressive ability of Trichoderma against downy mildew disease caused by *Sclerospora graminicola* in pearl millet. Sci Rep 7(1):1–11

Nepolean T, Gupta SK, Dwivedi SL, Bhattacharjee R, Rai KN, Hash CT (2012) Genetic diversity in maintainer and restorer lines of pearl millet. Crop Sci 52:2555

Onda Y, Mochida K (2016) Exploring genetic diversity in plants using high-throughput sequencing techniques. Curr Genom 17(4):358–367

Oumar I, Mariac C, Pham J-L, Vigouroux Y (2008) Phylogeny and origin of pearl millet (*Pennisetum glaucum* [L.] R. Br) as revealed by microsatellite loci. Theor Appl Genet 117:489–497

Parveen A, Mazhari BBZ, Rao S (2016) Impact of bio-nanogold on seed germination and seedling growth in *Pennisetum glaucum*. Enzyme Microb Technol 95:107–111

Passot S, Gnacko F, Moukouanga D, Lucas M, Guyomarc'h S, Ortega BM (2016) Characterization of pearl millet root architecture and anatomy reveals three types of lateral roots. Front Plant Sci 7:829

Poland JA, Rife TW (2012) Genotyping-by-sequencing for plant breeding and genetics. Plant Genpme 5:92–102

Rai KN, Murty DS, Andrews DJ, Bramel-cox PJ (1999) Genetic enhancement of Pearl millet and sorghum for the semi-arid tropics of Asia and Africa. Genome 42:617–628

Rai KN, Yadav OP, Rajpurohit BS, Patil HT, Govindaraj M, Khairwal IS et al (2013) Breeding pearl millet cultivars for high iron density with zinc density as an associated trait. J SAT Agric Res 11:1–7

Raipuria R (2012) STMS molecular marker-based diversity analysis in pearl millet genotypes varying for seedling salinity stress. M.Sc. Thesis submitted to Devi Ahilya Vishwavidyalay Indore, Madhya Pradesh, India, p 57

Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, Riera-Lizarazu O et al (2013) Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proc Natl Acad Sci USA 110:453–458

Reddy PS, Mallikarjuna G, Kaul T, Chakradhar T, Sopory SK, Reddy MK (2010) Molecular cloning and characterization of gene encoding for cytoplasmic Hsc70 from *Pennisetum glaucum* may play a protective role against abiotic stresses. Mol Genet Genomic 283:243–254. https://doi.org/10.1007/s00438-010-0518-7

Reddy PS, Reddy GM, Pandey P, Chandrasekhar K, Reddy MK (2012) Cloning and molecular characterization of a gene encoding late embryogenesis abundant protein from *Pennisetum glaucum*: protection against abiotic stresses. Mol Biol Rep 39:7163–7174. https://doi.org/10.1007/s11033-012-1548-5

Saha D, Gowda MVC, Arya L, Verma M, Bansal KC (2016) Genetic and genomic resources of small millets. Crit Rev Plant Sci 35:56–79. https://doi.org/10.1080/07352689.2016.1147907

Saïdou AA, Mariac C, Luong V, Pham JL, Bezançon G, Vigouroux Y (2009) Association studies identify natural variation at PHYC linked to flowering time and morphological variation in pearl millet. Genetics 182:899–910. https://doi.org/10.1186/1471-2164-14-159

Saïdou AA, Clotault J, Couderc M, Mariac C, Devos KM, Thuillet AC (2014) Association mapping, patterns of linkage disequilibrium and selection in the vicinity of the Phytochrome C gene in pearl millet. Theor Appl Genet 127:19–32. https://doi.org/10.1534/genetics.109.102756

Sankar MS, Satyavathi CT, Singh MP, Bharadwaj C, Singh SP, Barthakur S (2013) Genetic variability and association studies in pearl millet for grain yield and high temperature stress tolerance. Indian J Dryland Agric Res Dev 8(2):71–76

Sankar MS, Satyavathi CT, Singh MP, Bharadwaj C, Singh SP, Barthakur S (2014) Genetic diversity analysis for high temperature stress tolerance in pearl millet [*Pennisetum glaucum (L.) R. Br*]. Indian J Plant Physiol 19(4):324–329

Satyavathi CT (2019) Project coordinator review. ICAR-AICRP on pearl millet. Agriculture University, Jodhpur, India, pp 1–12

Satyavathi CT, Khandelwal V, Ambawat S (2019) R & D constraints and opportunities in pearl millet in India. One day national workshop on production, processing, value addition of nutricereals (millets) in India, held at CCS HAU, Hisar, India on 16 Sept 2019, pp 13–16

Satyavathi CT, Khandelwal V, Supriya A, Beniwal BR, Sushila B, Mahesh CK, Yadav SL, Sewaliya M, Meena H (2020) Pearl millet-hybrids and varieties 2020. ICAR-All India coordinated research project on pearl millet, Mandor, Jodhpur, India, p 37

Sehgal D, Skot L, Singh R, Srivastava RK, Das SP, Taunk J, Sharma PC, Pal R, Raj B, Hash CT, Yadav RS (2015) Exploring potential of pearl millet germplasm association panel for association mapping of drought tolerance traits. PLoS ONE 10(5)

Senthilvel S, Jayashree B, Mahalakshmi V, Kumar PS, Nakka S, Nepolean T, Hash CT (2008) Development and mapping of simple sequence repeat markers for pearl millet from data mining of expressed sequence tags. BMC Plant Biol 8(1):119

Serraj R, Hash CT, Rizvi SMH, Sharma A, Yadav RS, Bidinger FR (2005) Recent advances in marker-assisted selection for drought tolerance in pearl millet. Plant Prod Sci 8:332–335

Sharma PC, Singh D, Sehgal D, Singh G, Hash CT, Yadav RS (2014) Further evidence that a terminal drought tolerance QTL of pearl millet is associated with reduced salt uptake. Environ Exp Bot 102:48–57

Shinde H, Dudhate A, Anand L, Tsugama D, Gupta SK, Liu S, Takano T (2020) Small RNA sequencing reveals the role of pearl millet miRNAs and their targets in salinity stress responses. S Afr J Bot 132:395–402

Shivhare R, Lata C (2017) Exploration of genetic and genomic resources for abiotic and biotic stress tolerance in pearl millet. Front Plant Sci 7:2069

Shivhare R, Asif MH, Lata C (2020) Comparative transcriptome analysis reveals the genes and pathways involved in terminal drought tolerance in pearl millet. Plant MolBiol 103:639–652. https://doi.org/10.1007/s11103-020-01015-w

Siddaiah CN, Prasanth KVH, Satyanarayana NR, Mudili V, Gupta VK, Kalagatur NK, Satyavati T, Dai XF, Chen JY, Mocan A, Singh BP (2018) Chitosan nanoparticles having higher degree of acetylation induce resistance against pearl millet downy mildew through nitric oxide generation. Sci Rep 8(1):1–14

Singh P, Rai KN, Witcombe JR, Andrews DJ (1988) Population breeding methods in pearl millet improvement (*Pennisetum americanum*). L'Agron Trop 43:185–193

Singh SP, Satyavathi CT, Sankar SM (2014) Hybrid breeding in pearl millet: past and present status. In: ChawlaHS Shrotria PK, Jeena AS (eds) New paradigms in heterosis breeding: conventional and molecular approaches. Indian Agricultural Statistics Research Institute, New Delhi, India

Singh J, Reddy PS, Reddy S, Reddy M (2015) Molecular cloning and characterization of salt inducible dehydrin gene from the C_4 plant *Pennisetum glaucum*. Plant Gene 4:55–63. https://doi.org/10.1016/j.plgene.2015.08.002

Spindel J, Begum H, Akdemir D, Virk P, Collard B, Redona E et al (2015) Genomic selection and association mapping in rice (*Oryza sativa*): effect of trait genetic architecture, training population composition, marker number and statistical model on accuracy of rice genomic selection in elite, tropical rice breeding lines. PLoS Genet 11:1004982

Srivastava RK, Singh RB, Lakshmi V, Pujarula, Srikanth, Bollam, Pusuluri M, Satyavathi CT, Yadav RS, Gupta R (2020) Genome wide association studies and genomic selection in Pearl millet: advances and prospects. Front Genet 10. https://www.frontiersin.org/article/10.3389/fgene.2019.01389/full

Stich B, Haussmann BI, Pasam R, Bhosale S, Hash CT, Melchinger AE et al (2010) Patterns of molecular and phenotypic diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] from West and Central Africa and their relation to geographical and environmental parameters. BMC Plant Biol 10:216

Sun M, Huang D, Zhang A, Khan I, Yan H, Wang X, Zhang X, Zhang J, Huang L (2020) Transcriptome analysis of heat stress and drought stress in pearl millet based on Pacbio full-length transcriptome sequencing. BMC Plant Biol 20(1):1–15

Rajaram V, Nepolean T, Senthilvel S, Varshney RK, Vadez V, Srivastava RK, Shah TM, Supriya, A, Kumar S, Kumari BR, Bhanuprakash A (2013) Pearl millet [*Pennisetum glaucum* (L.) R. Br.] consensus linkage map constructed using four RIL mapping populations and newly developed EST-SSRs. BMC Genomics 14(1):159

Tarafdar JC, Raliya R, Mahawar HM, Rathore I (2014) Development of zinc nanofertilizer to enhance crop production in pearl millet (*Pennisetum americanum*). Agric Res 3(3):257–262

Tharanya M, Kholova J, Sivasakthi K, Seghal D, Hash CT, Raj B et al (2018) Quantitative trait loci (QTLs) for water use and crop production traits co-locate with major QTL for tolerance to water deficit in a fine mapping population of pearl millet (*Pennisetum glaucum* L. R. Br.) Theor Appl Genet. https://doi.org/10.1007/s00122-018-3094-6

Tiwari S, Lata C (2019) Genome engineering in rice: applications, advancements and future perspectives. In: Singh SP, Upadhyay SK, Pandey A, Kumar S (eds) Molecular approaches in plant biology and environmental challenges. Springer, Singapore, pp 323–337

Tiwari S, Lata C, Chauhan PS, Nautiyal CS (2016) Pseudomonas putida attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. Plant Physiol Biochem 99:108–117

Tiwari S, Lata C, Singh Chauhan P, Prasad V, Prasad M (2017) A functional genomic perspective on drought signalling and its crosstalk with phytohormone-mediated signalling pathways in plants. Curr Genom 18(6):469–482

Twomlow S, Shiferaw B, Cooper P, Keatinge JDH (2008) Integrating genetics and natural resource management for technology targeting and greater impact of agricultural research in the semi-arid tropics. Exp Agric 44:235–256

Vadez V, Hash T, Bidinger FR, Kholova J (2012) Phenotyping pearl millet for adaptation to drought. Front Physiol 3:1–386. https://doi.org/10.3389/fphys.2012.00386

Varshney RK, Shi C, Thudi M, Mariac C, Wallace J, Qi P et al (2017) Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. Nat Biotechnol 35(10):969. https://doi.org/10.1038/nbt.3943

Yadav OP, Rai KN (2013) Genetic improvement of pearl millet in India. Agric Res 2(4):275–292

Yadav RS, Hash CT, Bidinger FR, Howarth CJ (1999) QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet. In: Ito O, O'Toole J, Hardy B (eds) Genetic improvement of rice for water-limited environments. International Rice Research Institute, Manila, Philippines, pp 221–233

Yadav RS, Hash CT, Bidinger FR, Cavan GP, Howarth CJ (2002) Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought stress conditions. Theor Appl Genet 104:67–83

Yadav RS, Hash CT, Bidinger FR, Devos KM, Howarth CJ (2004) Genomic regions associated with grain yield and aspects of postflowering drought tolerance in pearl millet across stress environments and tester background. Euphytica 136:265–277

Yadav RS, Sehgal D, Vadez V (2010) Using genetic mapping and genomics approaches in understanding and improving drought tolerance in pearl millet. J Exp Bot 62:397–408. https://doi.org/10.1093/jxb/erq265

Yadav RS, Sehgal D, Vadez V (2011) Using genetic mapping and genomics approaches in understanding and improving drought tolerance in pearl millet. J Exp Bot 62:397–408

Yadav AK, Arya RK, Narwal MS (2014) Screening of pearl millet F_1 hybrids for heat tolerance at early seedling stage. Adv Agric 2014:17

Zaveri PP, Phul PS, Dhillon BS (1989) Observed and predicted genetic gains from single and mutli trait selections using three mating designs in pearl millet. Plant Breed 103:270–277

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. Plant Genome 1:5–20

Chapter 7
Genomic Designing for Abiotic Stress Tolerance in Foxtail Millet (*Setaria Italica* L.)

Sumi Rana, Lydia Pramitha, and Mehanathan Muthamilarasan

Abstract Small-seeded millets have gained significant research attention in recent years due to their climate-resilient nature. Among millets, foxtail millet [*Setaria italica* (L.) P. Beauv.] is considered as a model crop due to its rapid life cycle, small genome size, and self-pollinated nature. Its remarkable tolerance to abiotic stresses has invited researches on delineating the molecular machinery underlying tolerance and use the knowledge in developing elite cultivars that could withstand harsh weather and climatic conditions. However, crop improvement in foxtail millet has mostly been made through breeding strategies, but with the release of its draft genome sequence, several genes, QTLs, alleles, and markers were identified that regulate the tolerance traits. The effectual use of this information in crop improvement is yet to be realized as the progress made in foxtail millet research lags behind the major cereals. The genome editing approaches have recently gained importance as they enable precise editing of genes to achieve the desired phenotype. In foxtail millet, efforts are being invested in constructing vectors and optimizing experimental procedures for gene editing. In this context, the present chapter summarizes the progress made in identifying the genomic regions regulating abiotic stress tolerance and elaborates on how genomic designing could enable the development of climate-resilient varieties that could ensure food and nutritional security to the ever-growing population.

Sumi Rana, Lydia Pramitha contributed equally

S. Rana · M. Muthamilarasan (✉)
Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500046, Telangana, India
e-mail: muthu@uohyd.ac.in

L. Pramitha
School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences, Coimbatore 641114, Tamil Nadu, India

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7.1 Introduction

The last few decades have seen various global agriculture challenges due to the exploding population and severe climatic changes. This demands the development of better varieties that could withstand climatic aberrations and provides higher outputs. Climatic changes take a toll on the yield of several staple crops like maize, wheat, and rice. Although these crops are cultivated in more areas, they do not fulfill the major nutrient requirements of the ever-growing population and are easily affected by biotic and abiotic stresses (Lata et al. 2013). Millets, also known as nutricereals, are C_4 grasses that belong to the family Poaceae and are mainly known to yield smaller seeds. Finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), kodo millet (*Paspalum scrobiculatum*), pearl millet (*Pennisetum glaucum*), guinea millet (*Brachiaria deflexa*), tef (*Eragrostis tef*), and little millet (*Panicum sumatrense*), etc. are members of this family and used for consumption as food and feed (Dwivedi et al. 2012). They can withstand dry and semi-dry weather conditions, thrives in less fertile soil with minimal nutrients, needs less irrigation, and produces high nutrient containing seeds and hence, mentioned as climate-resilient crops (Muthamilarasan and Prasad 2015).

Among these, foxtail millet [*Setaria italica* (L.) P. Beauv.] has attracted the attention of researchers worldwide with its distinct traits, viz. morphologically small stature, self-fertilization, small and true diploid genome (∼423 Mb) with less repetitive DNA, high photosynthetic efficiency, rapid life cycle, prolific seed yield per plant, and tolerance to abiotic stresses (Lata et al. 2013; Table 7.1). These traits have made it a model crop to study genetic and molecular aspects of biofuel grasses and other C_4 crops (Lata et al. 2013). Li and Wu (1996) reported that it was domesticated in Northern China around 8000 years ago, considered one among the oldest grown crops, and is consumed as food and fodder in parts of Asia and Africa (Lu et al. 2009). It is one among the "Five grains of China" due to its higher protein and mineral content than rice, wheat, and maize (Austin 2006). Foxtail millet seeds contain 14–16% of protein, around 8% dietary fiber, 6% of crude fat, antioxidants, and minerals. The glucose release rate after foxtail millet consumption is slow due to the low glycemic index, making it suitable for diabetic patient consumption (Muthamilarasan et al. 2016). Furthermore, morphological features like thickened cell walls, small area of the leaf, alignment of epidermal cells, and compact and deep root architecture have aided the plant to achieve enhanced water use efficiency, nitrogen use efficiency, and even makes it tolerant to various abiotic stresses like heat, salt, and drought (Li 1997; Zhang et al. 2007; Lata et al. 2013; Diao et al. 2014). Foxtail millet holds the second position in world millet production wherein India contributes 32% of it (FAOSTAT 2005; http://faostat.fao/org/) (Table 7.2).

Table 7.2 Released varieties of foxtail millet popularly cultivated in different regions of India

Variety	Pedigree	Year of release and Institute	Maturity	Avg yield (Q/ha)	Cultivating zones	Key features
K3	Selection from SiA 2567	1989, TNAU	85–90	15–18	Tamil Nadu	Stay green nature
AK-132-1	Pure line	1990, APAU Ananthpur	82–85	18–20	Andhra Pradesh	Drought tolerance especially for Royalseema
Krisnadevaraya (SiA 2593)	Selection from SiA 326 × SiA 242	1993, Nandyal, Andhra Pradesh	85–90	20–22	Andhra Pradesh	High seed yield
TNAU 186	Co-5 × SiA 326	1994, TNAU	80–85	18–20	Tamil Nadu, Andhra Pradesh, Karnataka	Tolerance to Drought
TNAU 43	Co-5 × ISe 301	1994, TNAU	80–85	15–16	Tamil Nadu	High fodder yield
PS 4	SiA 2614 (0.2% EMS mutant)	1998, GBPUA and T Pantnagar UP	80–85	18–20	All states	Wider Adaptability
PRK 1	From Tehri selection	2000, GBPUA and T, Ranichauri	75–80	19–20	Uttar Pradesh	Early maturity
Meera (SR 16)	Pureline selection	2001,MPUA and T Udaipur	75–80	15–17	Rajasthan	Stay green resistant to downy mildew
Sri Lakshmi	Pureline selection	2002, ANGRAU Hyderabad	80–85	20–23	Andhra Pradesh	High seed yield
Prathapkangni 1 (SR 51)	Selection from local germplasm	2003, MPUA and T Udaipur	65–70	18–20	Rajasthan	Extra-early maturity with higher nutrition
TNAU 196	Co-5 × ISe 247		85–90		Tamil Nadu	Resistant to rust

(continued)

Table 7.2 (continued)

Variety	Pedigree	Year of release and Institute	Maturity	Avg yield (Q/ha)	Cultivating zones	Key features
		2005, TNAU Coimbatore		18–20		
Co 7	Co-6 × ISe 247	2005, TNAU coimbatore	85–90	18–19	Tamil Nadu	13.62–14.0% protein with fodder yield: 3.7–4.0 t/ha
HMT-100-1	RS-118 × PS 3	2008, ARS, Hanumanmatti and UAS Dharwad	90–95	20–25	Karnataka	High tillering, suitable for early and late sowing
SiA 3085	Pureline from SiA 2644	2011, RARS Nandyal ANRAU	80–85	20–30	All states	Resistant to blast and downy mildew
SiA 3156	Pureline from 2871	2012, RARS Nandyal ANRAU	85–90	20–25	Andhra Pradesh, Bihar, Gujarat, Karnataka, Madhya Pradesh, Tamil Nadu and Uttarakhand	Highly responsive to Nitrogenous fertilizers
Suriyanandi (SiA 3088)	Pureline from SiA 1244	2012, RARS Nandyal, ANRAU	70–75	20–25	All states	Non-lodging early maturing suitable for double cropping
RAU (Rajendra kauni 1–2)	Selected from local germplasm of laukaria, Raxaul and East champaran	2017, Rajendra Agricultural University, PAU	80–85	23–25	All states	Resistant against leaf blast, smut, brown spot, blight and downy mildew with high Fe content
DHF-t-109-3	Co-5 × GPUS 30	ARS, Hanmannamatti	86–88	29	Karnataka	Contingency planting with fodder yield of 52.30 q/ha

(continued)

Table 7.2 (continued)

Variety	Pedigree	Year of release and Institute	Maturity	Avg yield (Q/ha)	Cultivating zones	Key features
		and UAS Dharwad				
ATL 1	–	TNAU Athiyanthal	80–85	27.80	Tamil Nadu	Non-lodging, high yielding with synchronous maturity and fodder of 21.17 q/ha
CO 6	Hybrid derivative: CO 5 × ISE 301	TNAU	85–90	15–16	Tamil Nadu	Good grain quality with protein and fat rich contents
CO (Te) 7	CO 5 × ISE 248	TNAU	80–85	18–19	Tamil Nadu	Non-lodging and high yielding

The significant constraints to agricultural production are drought and irrigation; besides, even heat and salt stress significantly impact crop development and production (Ceccarelli and Grando 1996). Concerning the duration of exposure, severity, and time, abiotic stress may have acute and uncertain consequences on crop yield loss. Foxtail millet, being a climate-resilient crop, does not show a reduction in yield due to many abiotic stresses. In comparison to other cereals, the production of seed and yield efficiency of foxtail millet is less. Although rich in nutrients and tolerant to abiotic stress, the major constraints in the production of this crop are lack of awareness among the population. Hence, cultivation has been restricted to only a few parts of the globe. In many places, other crop cultivation has taken over it (Muthamilarasan and Prasad 2015).

On the other hand, the global population is increasing exponentially and might reach 10 billion by 2050. Furthermore, climate change as well as deteriorating soil fertility, expose the plants to several abiotic stresses viz. drought, heat, and salinity, imposing critical pressure on global agricultural productivity that further risks food and nutritional security (Kole et al. 2015). To compensate for climate changes, the input cost for cultivating major cereals has increased, which hikes the crops' price, making them unavailable to the poor. These major cereals do not meet the nutritional requirements and are susceptible to abiotic and biotic stress (Lata et al. 2013). From 2015 onwards, hunger and malnutrition are increasing in countries like the west of Asia, Africa, and Latin America, wherein the number accounts for 820 million people (1 out of 9 people). Due to the turbulence in climate and economy, and food insecurity, approximately 113 million people face acute hunger. Worldwide around 149 million children under the age of 5 are stunted, and Asia alone houses more than half of that number, i.e., 81.7 million (54.8%) (Global Nutrition Report 2020). Dependency on these major crops (rice, wheat, and maize) is one reason that malnutrition is still prevailing in a substantial level of the global population, mainly in Asia and sub-Saharan Africa (Deaton and Dreze 2009; Hirschi 2009). On the contrary, millets are resilient to heat, drought, and minimal nutrient conditions and can also meet the nutrient requirement of the ever-growing population. Despite having high nutritional values, foxtail millet has been neglected for all these years, but with the change in the climatic conditions, it is again being recognized and utilized.

7.2 Abiotic Stresses Affecting Foxtail Millet Cultivation

Foxtail millet is a crop that inhabits the natural ability to survive under various abiotic stresses. It has a higher water use efficiency than the C_4 maize, and its earlier duration makes it more feasible for the farmers to cultivate it in the rainfed zones (Zhang et al. 2007). Although foxtail millet is not affected by abiotic stresses to a greater extent, it is observed to face mild to severe damage depending on the cultivars. However, it faces a yield loss under water stress during its seedling and peak inflorescence stages. This has been brought under control by tracking the

drought-tolerant landraces using effective lab screening like polyethylene glycol (PEG) (Wen et al. 2005). Several drought-responsive genes have been molecularly characterized in foxtail millet, and this has been utilized to improvise its drought tolerance at sensitive stages (Muthamilarasan et al. 2014c). Transcriptomic profiling of saline tolerant lines provoked the role of glutamine synthetase and pyrroline-5-carboxylase in saline tolerance (Huang et al. 2013). The role of proline in buffering the existence of foxtail millet under saline conditions proceeded with acceleration in the breeding for salinity-tolerant lines (Veeranagamallaiah and Sudhakar 2017). Other abiotic stresses like cold tolerance, lodging, and waterlogging are being studied in breeding initiatives, and a cold-tolerant foxtail millet variety Liggu No. 26 was developed in China. In foxtail millet, a study of root characters for improving nitrogen use efficiency by Nadeem et al. (2018) and culm characters for enhancing non-lodging efficiency by Dwivedi et al. (2012) was conducted to minimize the influence of abiotic stresses, thereby resulting in expansion of area under cultivation of foxtail millet.

7.3 Molecular Mapping of Resistance/Tolerance Genes and QTLs for Abiotic Stresses

Efforts for mapping traits in foxtail millet began well before the knowledge of markers and genomic sequences. The exploration of collections of several foxtail millet accessions became a base for documenting its variable traits. Thus, traits such as plant height, inflorescence pattern, anthocyanin pigmentation, seed color, length of bristles, and anther and stigma color were used as morphological markers to identify true F_1. They also reveal a knowledge of the gene actions; for instance, non-glutinous nature was found to be dominant. Following these processes, the plant's cytology was studied, which served as a consequent marker for interspecific crosses. The chromosomal abnormalities, chromosomal morphology served as a tool for mapping the traits. Intrusive studies on cell metabolic actions in later stages developed the isozyme markers with cell-specific changes in their expression patterns to identify the genotypes and their cell-based mechanisms. These historical efforts significantly contributed to the initiation of mapping several traits in foxtail millet (Willweber-kishiomoto 1962; Zhang 1980; Radha et al. 2014).

7.3.1 Evolution of Marker Types: RFLPs to SNPs

The molecular markers are an essential tool that led to several identifications in foxtail millet genome-wide studies. Initially, in the foxtail millet, the RFLP probes detected the polymorphic changes in ribosomal DNA. Their amplification length differentiated these probes for ribosomal DNA in varied geographic regions. These

variations were used to classify the genotypes into three different sectors (Schontz and Rether 1998). Since the genomic sequence information for foxtail millet was not available during that period, the genetic diversity for conserved regions in the ribosome and mitochondria were employed to classify the genotypes molecularly. Such a study with *atp6* genes revealed three kinds of amplified products among the regions. They were designated as Type I, II, and III. The Type I and Type II were distinct due to the recombination between genes of *atp6*. The Type-I and II were found in Southeast Asian and Afghanistan races. Type I was distinctly present in India, while type-III was in China (Fukunago and Kato 2002). After RFLP, amplified fragment length polymorphism (AFLP) by Key Genes and random amplified polymorphic DNA (RAPD) dominant markers came into the scientific approach in foxtail millet during the 1990s. The historical mapping and analyzing diversity for varied regions with AFLP, RAPD, and nuclear RFLPs were successful in identifying the variations, and it grouped the Eastern Asian accessions from the Indian and Chinese vicinity (Fukunaga et al. 1997; Schonthz and Rether 1998; Fukunaga et al. 2002). These streams of markers also highlighted that the Indian accessions were highly variable. Following these molecular tools, simple sequence repeat (SSR) or microsatellite markers were utilized to cover the nine chromosomes in foxtail millet. One hundred ninety-three primer pairs from two libraries enriched for (GA)n and (CA)n in an F_2 population revealed 100 polymorphic primers across all the foxtail millet loci helped develop an SSR linkage map. This map was useful in integrating 81 newly developed SSR markers throughout the genome. The diversity with these markers by UPGMA proposed detecting 228 alleles classified in four clusters (Jia et al. 2009). These SSR markers were then predominantly used in several studies to detect the linkage disequilibrium (LD) values.

Along with these implications, the inter-simple sequence repeat (ISSR) markers in the comparative genomics of the genera in millets displayed the mutual relationship between coix and Setaria (Dvoráková et al. 2015). For identifying the exotic accessions in the Asian regions, the core of the worldwide collections was analyzed with 27 SSR and 4 Expressed sequence tag (EST) markers. These were used to analyze the parts of expressed genes that were complementary to the mRNA (Chander et al. 2017). After the completion of the sequence of the foxtail millet genome, there were several advancements with the identification of novel single nucleotide polymorphisms (SNPs) from mutants. The mutational approaches' beneficial mutants were compared with the wild types to detect the allele-specific traits, for example, Wp1 for chlorophyll pigmentation (Sun et al. 2019). Genotype-based sequencing approaches by GBddRAD helped identify markers linked to flag leaf width, 1000-grain weight, and yield (Jaiswal et al. 2019). Compared to the SSR markers, the SNP genotyping revealed a higher LD, which presents a higher diversity for SNP alleles across the genome of different accessions. These novel SNP alleles are now ravaging a lot of information in other species. These are now being used to extrapolate the genetic information so that genomic selection techniques could improve foxtail millet.

7.3.2 Mapping Populations and Their Use

A molecular base for any breeding program requires a mapping population. Considering foxtail millet, several linkage maps were constructed, and many quantitative trait loci (QTLs) were mapped. The mapping studies for foxtail millet predominantly included F_2 populations. Starting from Sato et al. (2013), who initially employed SSR markers for identifying the gene responsible for spikelet-tipped bristles, the rest of the breeders also adopted this as a base population for QTL mapping. Maintaining an F_2 population with higher segregation serves as a base for mapping several key traits, and Fang et al. (2016) used around 10,598 SSR markers to map 29 QTLs. Further, QTL-sequencing by bulk segregant analysis for heading dates with three bulks viz, early heading, late heading, and extremely late heading were used to tap the QTL loci for heading (inflorescence initiation) in this crop (Yoshitsu et al. 2017). A high-density genetic map and QTL analysis with F_2 population using RAD-sequencing detected 11 major QTLs for eight agronomic traits (Wang et al. 2017). This projects that the F_2 population has been commonly used for mapping traits in foxtail millet and a genome-wide bin analysis with SNP-based markers also developed 11 significant QTLs for eight traits, which could further be utilized as markers (Wang et al. 2019).

Following the F_2, recombinant inbred lines were successively used in the foxtail millet to map the agronomic traits (Ni et al. 2016). Around 493 recombinant lines were utilized for developing a high-resolution bin map that facilitated high-density SNP-based markers (Zhang et al. 2017). Similar re-sequencing of 184 RILs was developed to identify *sd1* genes beneath the plant height in foxtail millet (Ni et al. 2017). The significant advantage of using these recombinant inbred lines is that it is desirable to screen them across locations. F_2 being segregating population results in some difficulties in isolating genetic and environmental variations, and by using 164 RILs across locations, three stable QTLs were observed in chromosomes 3, 6, 7, and 9 by Liu et al. (2020). There were no attempts to map traits with near-isogenic lines as there were not much backcross programs conducted to introgress traits for their improvements. Since foxtail millet had no such demands for trait-specific developments such as disease/pest resistance, future back cross programs could be effected for endemic zones where its cultivation is affected. Similarly, double haploid techniques have not been incorporated due to the lack of proper tissue culture strategy in this crop. Modified populations like pseudo test cross performed in several other crops have some limitations to be encountered as it is a highly self-pollinated crop.

7.3.3 Mapping Approaches and Maps of Different Generations

Different mapping software were employed to identify QTLs and their LOD values. Wang et al. (1998) developed the first linkage map in foxtail millet. Following the same procedure, Doust et al. (2004, 2005) and Jia et al. (2009) adopted Mapmaker v3 in constructing genetic linkage maps in foxtail millet. Later approaches by Ni et al. (2016, 2017) used the MSTmap software package. Following these techniques, MSTMap was also used in performing composite interval mapping (CIM) in the construction of linkage maps (Wang et al. 2017; Zhang et al. 2017). Haldane mapping function was later used in RIL populations to mark the position of the markers to its linkage groups using CarthaGene v1.2.3. (Yoshitsu et al. 2017). Other than CIM, multiple QTL mapping for linkage map was also conducted by R package named one map (Liu et al. 2020). Besides, MSTmap software and JoinMap 4.0 using Haldane and Kosambi mapping function, respectively, were used to obtain precise maps for the mapping of several loci in foxtail millet chromosomes (Sato et al. 2013; Fang et al. 2016; Zhang et al. 2017; Wang et al. 2017; Ni et al. 2017). MSTMap is the most used software for linkage map construction in foxtail millet in comparison with all the packages.

The initial framework for constructing a genetic linkage map in foxtail millet was performed by Wang et al. (1998) with an intervarietal cross for flower color. This map was about 964 cM and was constructed with the help of cytogenetic tools by implying trisomic lines and polymorphic RFLP probes. It depicted chromosome VIII to be the shortest and chromosome IX as the longest chromosome. This map was used as a base by the followers to construct future linkage maps that helped mapping the genes like *tb1* in the chromosomes V and VI.

Further, 27 new RFLP probes from maize were mapped to this linkage map by Doust et al. 2004 and as a continuation by Doust et al. (2005), the synteny of rice and other millets on foxtail millet were included to develop a high-density linkage map for the inflorescence patterns. After this, 101 SSR markers were mapped on the nine chromosomes of foxtail millet, wherein chromosome 9 had the densest coverage of markers. This map was confined to the previous map developed by Devos et al. (1998), which was initially constructed with 20 RFLP probes. Later with 86 SSR primers, a maximum coverage in the genome was attained (Jia et al. 2009). Hence, this map was continually refined to a high-resolution map by Sato et al. (2013), who further enriched this map with an additional 26 SSRs and 87 transposon display markers. Altogether finally, this refined map consisted of 13 linkage groups.

With the availability of genome sequence information, a high density and high-resolution map with 1058 SSR markers detecting 1035 loci were constructed, and this was the most saturated map among all the previous foxtail millet studies (Fang et al. 2016). This broader map could be used for functional gene mapping and map-based cloning in the near future. Inclusively, high-density linkage map for 14 agronomic traits under different photoperiods (Zhang et al. 2017), linkage maps for

nine phenological traits (Ni et al. 2017), high-density map with 10016 SNP markers (Wang et al. 2017), linkage map by advanced RAD-seq with SNP spanning (Wang et al. 2019) and also binary map construction including 3413 bin markers were developed in foxtail millet. These maps were saturated and more advanced than the RFLP linkage maps probes.

7.3.4 *Enumeration of Mapping of Simply-Inherited Stress Tolerance Traits*

Different mapping functions were used in developing QTLs and linkage maps that could be used in future molecular breeding. The Kosambi function was initially used for mapping the RFLP probes. The recombination fractions were converted into centimorgans for mapping (Wang et al. 1998). For tapping the genes responsible for plant height and axillary branching in foxtail millet, composite interval mapping was employed later (Doust et al. 2004). Following this, Doust et al. (2005) used three-point linkage analysis to map the markers. Various authors preferred different mapping techniques in constructing genetic maps, and Kosambi function was more used than Haldane (Jia et al. 2009; Fang et al. 2016; Ni et al. 2017). Regarding this, the Haldane mapping function in the construction of genetic maps was implied for agronomic parameters (Yoshitsu et al. 2017; Wang et al. 2017). Composite interval mapping and maximum likelihood calculations were done to segregate markers across nine chromosomes done by Zhang et al. (2017) and Wang et al. (2019). Wang et al. (2019) also used the software Biomercator v3 for integrating selected maps into a reference map by using the applications InforMap and ConsMap in Biomercator. Recently, high-throughput sequencing technologies with SNPs facilitated a desirable multiple QTL mapping with bin markers. These bins are calculated from the recombination breakpoints in which the genotype changes from one type to the other along the chromosomes. This was used as a skeleton to construct a skeleton bin map for the nine chromosomes (Liu et al. 2020). This presents the thrust for developing high-resolution maps with SNPs in the future.

7.3.5 *Framework Maps and Markers for Mapping Stress Tolerance QTLs*

From the above sections, it is noteworthy that several maps from cytogenetic to high-resolution SNP maps have been generated in foxtail millet. The linkage map constructions proclaim the use of all predominant markers starting from RFLP to cleaved amplified polymorphic sequences (CAPS), transposon display, SSRs, and SNPs obtained by different genotyping techniques. Framework maps were initially constructed by Wang et al. (1998), Doust et al. (2004), and Jia et al. (2009). The

Table 7.1 Key morphological and cytological observations in different species of *Setaria*

S No	Species	Key features
1.	*Setaria italica*	Known as Italian millet, yellowish and elliptical seeds, non-glutinous and glutinous races, diploid ($2n = 18$), annual with no rhizome, wide and longer leaves, plant height, low number of bristles, white—brown anthers, white stigma, higher germination, non-shattering and abundant spinules
2.	*Setaria viridis*	Green millet, non-glutinous in nature, more number of primary branches, spinous, inflorescence with bristles, dark anthers with white stigma, medium stature, narrow and shorter leaves, diploid annual with no rhizome, apical spikelet, dark brown elliptical seeds, lesser seed yield and fertility, dormancy, seeds shattering after maturity, lower germination%, var: *Setaria viridis polystachys* adapts in stony coastal areas
3.	*Setaria faberii*	Morphologically similar to *viridis* except that it is twice larger in plant and seed size, white stigma, higher seed fertility, early flowering, dormancy, tetraploid (4x) annual with no rhizome, color of the plants vary from green to purple, Different genome than the formers
4.	*Setaria verticillata*	Adapts to subtropical regions, whole plant is green, it has intermediate features between *viridis* and *faberii* with an erect panicle, dark brown elliptical seeds, smallest seeds, retrose spinules in the spinules is distinct and panicles get entangled, tetraploid (4x) annual with no rhizome, no dormancy with highest germinability, larger spinules and cells than diploids
5.	*Setaria pyconoma*	Appears like a natural hybrid between *italica* and *viridis* with seed colors from light brown to brown, wide to narrow leaves, spherical-elliptical seeds, white stigma, lesser and small spinules and diploid annual with no rhizome
6.	*Setaria lutescens*	Tetraploid (4*x*), annual with no rhizome, spike like inflorescence, purple stigma, glutinous/waxy, inner and outer glume transversely rugose, sterile apical spikelet, largest bold seeds in *Setaria*, yellowish to purple bristles with larger stomata, panicles are whitish with yellow-purple bristles, panicle length varies from 4 to 14 cm in var. *longispasca* with dormancy in seeds, very few spinules in veins, second floret has no pistil and it flowers 3–7 days later than first
7.	*Setaria pallide-fusca*	Octaploid (8*x*), annual with no rhizome, found mostly with lutescens. Except the cells and plant size is larger than lutescens except for seed. Similar inflorescence as that of lutescens with waxy nature. It has a prostrate panicle with reddish purple bristles with seed dormancy with very few spinules in veins
8.	*Setaria chondrachne*	Tetraploid (4*x*) and perennial with rhizome, inflorescence is panicle like a tassel of maize, non-waxy, purple stigma, inner and outer glumes have smooth surface with larger stomata, long spines between veins
9.	*Setaria excurrens*	Octaploid (8*x*) perennial with rhizome, non-waxy, white/purple stigma, seed are long with smaller width, panicle like inflorescence with minimum number of branches, inner & outer glumes have a transversely rugose surface long narrow leaves, cultivated in Japan with seed dormancy

first reference genome sequence of foxtail millet was developed in 2012 for the Yugu1 variety, which was employed in several mapping techniques. The segregation of the markers and their polymorphic pattern were the main criteria involved in genetic linkage mapping in foxtail millet.

7.3.6 Depiction of QTL Maps

The LOD in a QTL mapping determines its exact positions in a chromosome, and such maps have been constructed by the authors who conducted a QTL analysis as mentioned above. A LOD score above three is considered to be a major QTL, which can be reliable in molecular breeding, and such QTLs have been enlisted in Table 7.3. The chromosomal positions for the traits, including plant height, heading date, flag leaf length, and width, have attained peaks for chromosomes 5 and 2, respectively. It could be understood that based on the studies carried out by Ni et al. (2017), the loci positions for these traits are present in chromosomes 5 and 2.

7.4 Marker-Assisted Breeding for Resistance Traits

7.4.1 Germplasm Characterization and DUS

Identifying and classifying the germplasm forms the primary dataset for effecting crop improvement in a population. Foxtail millet being a predominant millet across the world, requires an observation of the entities collected around the globe. Reddy et al. (2006) carried out such work in ICRISAT with 1535 accessions collected from 26 countries. They characterized the entire group, and it was found that the Indian accessions had the maximum variability for foxtail millet while the least was for the accessions from Russia. The accessions collected from each country were classified into races and sub-races based on their inflorescence pattern, and the collections from India had all the races except for the sub-race fusiformis. These observations depicted a higher diversity in Indian accessions, like their date of flowering, inflorescence exertion, and plant height. The Chinese accessions were dwarf and decumbent, resembling the *S. viridis* species, while Indian accessions were taller and erect. The majority of the accessions were taller with medium flowering duration, longer inflorescence, green-leafed, with yellow-colored seeds. By grouping the accessions, it was found that the majority of them belonged to the race *indica* and *maxima*. These accessions were further exchanged with several countries like Africa, America, and Europe for their distinct evaluation.

Regular characterizations in different collections across countries is a laborious process that also involves the possibility of experimental errors. To overrule, this a subset of such collections representing the overall population is essential to preserve

Table 7.3 Details of trait-wise QTLs identified in foxtail millet genome

Trait	QTL	Position	LOD	Phenotypic variance (%)	Gene action	Parental effect	Reference
Tiller number	Till1	Chromosome 5	>4	28.10	Epistasis	male	Doust et al. (2004)
Axillary branching	SQUAX 1	Chromosome 6	>5	24.80	Epistasis	male	
Number of spikelets	SPK	Chromosome 9	=	23.50	Additive	Female	Doust et al. (2005)
Bristle number/ primary branch	BR	Chromosome 8	=	19.10	Additive	Female	
Panicle length	qPL 6.1	Chromosome 6	5.02	13.30	Additive	Male	Fang et al. (2016)
Straw weight/ plant	qSWP 1.1	Chromosome 1	5.45	14.30	Additive	Female	
Node number of main stem	qNMS 1.1	Chromosome 1	4.93	13.10	Additive	Female	
Tiller number	qTN 5	Chromosome 5	30.87	25.72	Additive	Female	Zhang et al. (2017)
Plant height	qPH5	Chromosome 5	70.06	43.94	Additive	Male	
First main Internode diameter	qFMID 9.1	Chromosome 9	4.80	15.50	Additive	Female	Wang et al. (2017)
Second main internode diameter	qSMID9.1	Chromosome 9	4.11	13.50	Additive	Female	
Plant height	qPH5-2	Chromosome 5	31.90	30.52	Additive	Male	Wang et al. (2019)
Panicle diameter	qPD5-2	Chromosome 5	12.7	10.72	Additive	Male	
Panicle weight	qPW5-1	Chromosome 5	10.8	10.74	Additive	Male	
Pericarp colour	qPC7-2	Chromosome 7	16.0	18.54	Additive	Male	
Grain weight/ plant	qGWP9.3	Chromosome 9	4.60	12.20	Additive	male	Liu et al. (2020)
Grain weigh/ plant	qGWP6.1	Chromosome 6	4.22	11.20	Additive	female	

the complete genetic richness of conserved germplasm. Plant genetic resources portray the development of core, mini-core, and reference set collections in conserving the overall variability. The core collections are observed to have a population size of 10% of the whole base population. They are entitled to represent the entire genetic base of the population. For manipulation across locations, a more desirable mini-core concept was developed, and this is a representation of the entire core subset that is formed. The mini-core comprises 1% of the core collection with maximum representation for all the traits. Favoring trait-specific breeding programs, a reference set/collection is also formulated from the base. This is formed so that the overall variability of the base population for a particular trait is represented. They have a population that follows a normal distribution for a specific trait from the base (Upadhyaya et al. 2009a, b; Pramitha et al. 2020).

These conservation techniques are also adopted in conserving the foxtail millet genetic diversity. ICRISAT is one of the organizations that preserve worldwide collections for foxtail millet, and around 1474 accessions from 23 countries are being characterized and conserved. Initially, a core collection with 155 accessions representing the entire 1474 accessions was developed to ease handling and multi-environmental trials. For the formation of this core initially, a principal component analysis separated the base accessions into 29 clusters, and 10% of the accessions from each cluster constituted this core. X^2 and Shannon-Weaver diversity index indicated the null difference between the core and the base set. The accessions represented a maximum diversity, and they were grouped based on 12 qualitative and 11 quantitative traits following a continuous variation with descriptors of *S. italica* and *S. pumila* from IBPGR,1985. The core was further stratified based on its races, and similar to the base population, a maximum variability was observed in *indica* and *maxima* (Upadhyaya et al. 2008). These collections could be improvised later with reference set screening for nutritional traits, abiotic and biotic stress tolerances in the future.

As a support to international characterizations, nation-wide diversity studies concentrating on yield contributing traits on foxtail millet were performed in India (Upadhyaya et al. 2008). Nirmalakumari and Vetriventhan (2010) conducted initial characterizations for 741 accessions from Tamil Nadu Agricultural University for seven traits. This presented the highest heritability and genetic advance for yield with minimum values for days to 50% flowering. Also the traits, days to 50% flowering, plant height, number of tillers, number of productive tillers, panicle length, and days to maturity were identified as possible indicators for higher yield by its correlation. Parallel to these characterizations, China is a water-scarce country and has predominant importance for foxtail millet cultivation from its domestication era. A base population of 3356 accessions was initially studied, and these vast collections were from the Northwest, Northeast, and Northern parts of China. From this base, 128 accessions were sub-setted and subjected to 79 genome-wide SSR markers for characterization. The population structure indicated the highest diversity among the panel and clustered the accessions into six groups. The second group had unique geographic origins with a higher intra-population diversity from central and southern Shanxi province, which could be further utilized in breeding

programs. Thus, it revealed that the clustering pattern was dependent on spring and summer type adaptations in China's foxtail millet collections (Liu et al. 2011). Studies were made in a foxtail millet for two seasons to enumerate the environmental influence, and a higher yield of the accessions in summer was recorded (Brunda et al. 2015).

Comprehensive analysis with core and reference populations was later conducted in China with the base population of 27,509 accessions. From this, China has identified several drought-tolerant, rust-resistant, and high protein compassing genotypes. This developed core and reference set in china have also revealed the inheritance of many morphological characteristics. For example, seed color was found to be controlled by three loci (*BBIIKK*). *BB* causes gray, *II* enhances color, and *KK* produces yellow seeds. The order of dominance of seed color is light yellow > red > white > yellow color. The waxy trait in the collections was dominant (chromosome 1 and 9), and the disruption of the GBSS1 gene causes it. Similarly, long bristles, purple bristles (chromosome 4), orange anther color (chromosome 6), purple leaf sheath (chromosome 7), palmate panicle, and plant height (chromosome 3) were dominantly inherited in the foxtail millet germplasm collections, and they were successfully used as morphological markers to identify hybrids (Diao and Jia 2017). Thus, the concept of sub-setting a larger population was well-utilized in China.

The utility of germplasm relies on its variability; thus, screening a more extensive base population earlier will be more effective and helpful in developing core samples for vast collections. Core collections for foxtail millet were formed only in India and China. Both the countries have documented the passport data of the accessions collected across countries. In India, the AICRP project characterized 1312 germplasm accessions under the small millets improvement initiative. This described the importance of documenting the quantitative traits like days to 50% flowering, peduncle length, flag leaf length, and width among the 37 clusters formed by D^2 analysis. The array of observations revealed a higher variability with a negative correlation for plant height and flowering with yield. In contrast, the traits—number of basal tillers and peduncle length were positively associated with increasing the yield pattern (Nandini et al. 2018). These accessions could be further improvised by formulating a core/reference like China for facilitating trait-specific breeding in India.

On par with morphological characterization, qualitative characterization for macro- and micronutrients in foxtail millet accessions also presented a higher variability for the content of protein, carbohydrate, calcium, magnesium, iron, zinc, copper, manganese, and other minerals. Superior genotypes were characterized and identified for forwarding to bio-fortification trials (Pavani et al. 2019). This states the overall efforts in characterizing the germplasm accessions across the world for various traits in foxtail millet. These characterizations require criteria to be adopted, and these are framed by international and national organizations for germplasm conservation to avoid ambiguities. The varietal identification and germplasm documenting should be based on DUS descriptors. Such a descriptor for characterizing foxtail millet plays a major role in protecting breeder's rights under the Protection

of Plant Variety and Farmers Right Act (PPVFR Act—2001). DUS refers to the distinctiveness, uniformity, and stability of the accessions characterized. They are used to identify the uniqueness of a variety. In any admixtures or off-types, the descriptors are used as unique indicators to identify original accessions from the admixtures. The record of the observations for DUS requires some guidelines to be fulfilled. This screening should be done in two independent similar growing seasons across two locations under PPVFRA. Seeds weighing 250 g with maximum germination percentage is sent for notifying a variety by an applicant. Each observation should include the mean values of 40 plants, and the color characteristics would be recorded based on RHS (Royal Horticultural Society) color charts.

DUS characterization on 223 genotypes was performed in a reference set aiming at trait-specific improvement. These observations rendered a maximum variability for growth habit, pubescence, pigmentation, leaf attitude, inflorescence shape, grain color, and grain shape. Sixteen qualitative traits from DUS were used for documenting the variability in the accessions in India. These highly variable traits could be utilized to recognize the release of a variety in the future (Banu et al. 2018). Following this study, Amarnath et al. (2019) also utilized DUS descriptors to classify the accessions into different clusters by D^2 analysis. These descriptions of an accession favor the fingerprinting of a variety to be released, and this preserves a breeders' rights to register the nature of the varieties released. Further, this avoids the misuse of varieties by any foreign authority without any prior information, and thereby a proper documenting of the overall variability in *Setaria* will be done with a pedigree record.

7.4.2 Marker-Assisted Gene Introgression

Foxtail millet is a climate-resilient crop with a higher photosynthetic efficiency due to its C_4 mechanism. Being a self-pollinated crop in nature, it has several constraints in exploiting its heterotic potential. Molecular markers play an essential role in characterizing and mapping genetic diversity across populations. At the same time, there are not many studies yet conducted in transferring trait-specific genes by introgression in foxtail millet. Foxtail millet naturally bestows a potential for all the favorable genes within its genome, and molecular mapping of QTLs for these traits are continuously studied from RIL populations. Recently stable QTLs for yield components and straw weight were mapped to chromosomes 3, 6, 7, and 9 of foxtail millet (Liu et al. 2020). Further, nine QTLs for drought (Qie et al. 2014), yield components related to panicle (Odonkor et al. 2018), and other QTLs for agronomic traits (Ni et al. 2017) were being explored in RIL, F_2, and F_7 populations.

There is not much necessity for improving the yield concerns in foxtail millet by introgression as a larger extent is being achieved from the natural variability by hybridization. Introgression could be carried out for some specific abiotic/biotic stress tolerance in epidemic zones where foxtail millet is significantly affected by stresses. This requires the identification of particular donors for various traits by

genetic screening and molecular characterization. Several donors for drought tolerance, saline tolerance, flooding tolerance, and non-lodging efficiencies could be identified from comprehensive phenotyping and genotyping. These pre-breeding lines with proper background and foreground markers could be employed in marker-assisted backcross programs to improvise the genetic makeup of foxtail millet. The recurrent parent and the donors selected should also encompass a broad genetic base to avoid genetic bottlenecks in the future.

7.4.3 Gene Pyramiding

Gene pyramiding is the process of combining several desirable genes in a single cultivar to develop an elite variety. Multi-flexing in foxtail millet cultivars has not yet been initiated. Foxtail millet is a hardy crop with not much pre- and post-harvest losses in cultivation. It is almost not infested by any pests and diseases due to its spontaneous host plant resistance. The bristled inflorescence and spines in leaves are non-preferable for any insect attacks, and the higher phenol metabolites in it provide a natural tolerance against diseases. This is more yielding than other mainstream cereals with a minimum cost of cultivation and higher profit. This happens to be a primary reason for its lesser scope in gene pyramiding techniques. However, this crop is better suited as a reference for several millet species, which are now being explored.

7.4.4 Limitations and Prospects of MAS and MABCB

Marker-assisted selection (MAS) and marker-assisted backcross breeding (MABCB) are advanced techniques that brought about several elite cultivars in mainstream cereals and pulses subjected to severe yield losses. These are now utilized in biofortification trials for improving nutritional traits across crop species. In foxtail millet, these techniques have not been exploited yet. This may be because there was not much necessity in altering or introducing any trait that is absent in it. This crop is being known to possess weedy features and naturally strive in diverse conditions with a high yield. However, if this need arises in the future, these techniques could be successfully employed as the complete genomic sequence information is available in several databases like Gramene (www.gramene.org/). But coming to the backcross, effecting a crossing technique will be a limitation in this crop due to its small florets and lower seed setting capacity. This was why the breeders opted for pure line selection and mutational breeding in developing cultivars in foxtail millet. This has to be overcome with alternate strategies so that several breeding introgression libraries as that of rice could be developed in the future.

MAS has been carried out effectively across populations, followed by mapping the trait loci in chromosomes. With the advent of SNP genotyping, several advanced techniques have been used to identify suitable parental lines. But this lacks proper introgression techniques because until now, only the F_2 derived progenies are used as mapping populations. Advanced techniques like GWAS and pyramiding has to be further exploited in varieties for future demands. Other than hybridization difficulties, these techniques could possibly be utilized in the future for improving foxtail millet cultivation in different agro-ecological zones.

7.5 Map-Based Cloning of Resistance/Tolerance Genes

Map-based cloning, also known as positional cloning, helps decipher the genetics of any mutant phenotype using known markers present in the genome. *siago1* mutant of Foxtail millet was developed by ethyl methanesulfonate (EMS) treatment of the Yugu1 variety, which showed many developmental abnormalities like thin and curled leaf edges, dwarfed stem, and panicles, and so on. Analysis of the *siago1* mutant by map-based cloning revealed that the anomalies were caused by deleting 7 bp in the C-terminus from the *SiAGO1b* gene and transversion (C–A). RNA-seq expression data comparison of wild type and SiAGO1b mutant showed that 1598 genes were differentially expressed, which may have a role in growth and development, abiotic stress response, cell death, and energy metabolism, etc. BiFC and Y2H assay revealed that the mutated region contained the functional motif for the interaction of *SiHYL1* and *SiAGO1b* (Liu et al. 2016). Li et al. (2016) performed map-based cloning in yellow-green leaf mutant, viz. *siygl1* that revealed *SiYGL1* is responsible for the phenotype of the mutant. The mutant had a lesser accumulation of chlorophyll (Chl) with reformed ultrastructure due to change in the amino acid phenylalanine to leucine around the ATPase-conserved domain. Gene expression analysis of SiYGL1 and wild type revealed that SiYGL1 regulated genes like *DEG2* (development thylakoid), *LHCB1,* and *rbcL* (photosynthesis), and *SRP54CP* (chloroplast signaling).

Zhang et al. (2018) isolated the *sistl2* (*Setaria italica* stripe leaf mutant) mutant of foxtail millet. They performed map-based cloning that revealed *SiSTL2* (encodes DCD: deoxycytidine monophosphate deaminase protein) to be the causal agent of the mutant phenotype. In comparison to the Yugu1 variety, *sistl2* showed slowed progression in the cell cycle, leaves with stripes, dwarfed stature, and abnormal ultrastructure of the chloroplast. The *SiSTL2* expression patterns, in response to low CO_2, match the expression pattern of C_4 genes. Silencing of *SiSTL2* showed decreased 13C leaf content, and during photosynthetic carbon fixation, it increased the DEGs. In another study, Zhang et al. (2018) have performed gene expression analysis and characterized the phenotype of a yellow-green leaf mutant of foxtail millet (*siygl2*) variety Yugu1. The result showed that SiYGL2 is involved in the progression of leaf senescence, regulates the content of chlorophyll, and also regulates the function of PS II. Tang et al. (2019) studied the ribonucleotide reductase

(RNR) in *sistl1* mutant of foxtail millet that showed phenotype with striped leaf and reduced chloroplast accumulation due to the substitution of glycine to glutamate in the *SiSTL1* protein. They also showed that *SiSTL1*, encodes the larger subunit of RNR, is essential for growth, the progression of the cell cycle, and the biogenesis of chloroplast in foxtail millet. Map-based cloning is a time-consuming method and is also tedious, but with the availability of the reference genome of the foxtail millet, this process can be made faster. Map-based cloning can also be accompanied by speed breeding method and double haploid culture to get quicker results (Watson et al. 2018).

7.6 Genomics-Aided Breeding for Resistance/Tolerance Traits

7.6.1 Details of Genome Sequencing

In 2012, two independent teams, Beijing Genome Initiative (BGI), China, and the United States Department of Energy Joint Genome Institute (USDOE-JGI), USA, sequenced the genome of foxtail millet and its wild ancestor green foxtail (Bennetzen et al. 2012; Zhang et al. 2012). The team BGI China has used the inbred foxtail millet strain "Zhang gu" and green foxtail strain "A10". They have used the whole genome shotgun combined with next-generation sequencing for the assembly of foxtail millet genome. Insert size of 170 bp–40 kb was used to create DNA libraries followed by sequencing with Illumina second-generation sequencing. The raw data output was 63.5 Gb, which was filtered down to about 40 Gb clean reads that served as an input for SOAPdenovo for genome assembly. Generation of contigs by *de Bruijn* graphs and post-gap filling, the contig N50 was found to be 25.4 Kb, 90% of which was present in 16,903 contigs. Furthermore, scaffold N50 was of the size of 1.0 Mb, and 384 Mb (90% of scaffolds) was seen to be in 439 longest scaffolds. Cytogenetic methods and k-mer analysis estimated the genome size to be approximately 490 Mb and 485 Mb, respectively, with 6.6% gaps (28 Mb) wherein the scaffolds extended over about 86% of the complete genome. Transposable elements acquired ~46% of the draft genome when a complete repeat annotation was performed, which included retroelements (~133.6 Mb) like LTR (*Gypsy, Copia,* and others), LINEs and SINEs, and DNA transposons (~39.7 Mb) comprising tandem repeats like CACTA, *h*AT, Helitron, Stowaway, Tourist, etc. (Zhang et al. 2012). The team from USDOE-JGI used inbred foxtail millet strain "Yugu1" and green foxtail for sequencing with ABI3730xl capillary sequencer and Illumina Genome Analyzer II platform, respectively. Insert size of 121 Kb, covering almost 12X of the genome, was used to develop the BAC library, further used for BAC-end sequence analysis. The sequence data generated was 4 Gb that showed the genomic sequence of the "Yugu1" strain comprised of

396.7 Mb across nine chromosomes and 327 scaffolds of 4.2 Mb that covered around 80% of the genome (Bennetzen et al. 2012).

7.6.2 Gene Annotation

In foxtail millet, about 38,801 genes were found using consolidated annotation methods. Homologs of these genes with known functions were retrieved by mapping them against various protein databases like GO, InterPro, KEGG, TrEMBL, and SwissProt. Overall, 30,579 genes were annotated, while 8220 genes could not be annotated using the homolog function information. According to the gene ontology annotation, around 79% of the identified genes have homologs in public databases with well-defined functions. The transcriptome analysis of tissues from the root, spica, leaf, and stem showed expression of around 82% predicted genes in them. The average of 4.3 number of exons per gene is present. Besides, the intron's average length was 442 bp, whereas the average length of the exon was 256 bp. The prediction of 1367 pseudogenes genes showed that they could be retrotransposed, duplicated, or be unclassified. The non-coding RNA gene prediction in the foxtail millet genome revealed that chromosomes 1, 7, 8, and 9 contained huge clusters of rRNA genes that accounted for 99 in number. In contrast, the rest non-coding RNA genes showed limited chromosomal distribution. The non-coding RNA genes contained 704 tRNA genes; 382 snRNA genes included HACA-box, CD-box, and splicing, and 159 miRNA genes (Zhang et al. 2012).

As angiosperms show a higher degree of gene conservation, Bennetzen et al. (2012) annotated the completely assembled whole-genome sequence of foxtail millet according to the reports available in other grasses and model plant—Arabidopsis. Annotation revealed 35,472 primary transcripts of protein-coding genes along with 5128 alternate transcripts and 11% of which can be putative candidates for foxtail millet study due to their novelty. Annotated genes have an average intron length of 163 bp and an average exon length of 135 bp. The protein was seen to contain an average of 329 amino acids. Foxtail millet has around 40% of TEs, which is comparatively less than the other grasses, making it a model crop. The study of C_4 photosynthesis pathway genes viz. *PEPC, PPDK,* and *MDH* with maize and sorghum orthologs showed a higher conservation degree. Six clusters of drought-associated genes were found during Setaria genome analysis, with a higher number of drought-tolerant species, like Setaria and sorghum, than the drought-susceptible species like rice and maize (Bennetzen et al. 2012; Muthamilarasan and Prasad 2017). Functional studies in foxtail millet with putative candidate genes like multi-antimicrobial extrusion protein, NADH oxidase, plant lipid transfer protein, Aldo/keto reductase, AMP-dependent synthetase/ligase, and glutathione S-transferase, could aid in unraveling the genetic rationale for stress adaptation in them. The analysis of C_4 photosynthetic genes, namely *PEPC, PPDK,* and *MDH* showed higher conservation with sorghum as well as maize. In contrast,

the malic enzyme isoform on foxtail millet did not show any conservation. This result could be exploited to study C_4 photosynthesis pathway evolution (Bennetzen et al. 2012; Muthamilarasan and Prasad 2017).

7.6.3 Impact on Germplasm Characterization and Gene Discovery

The release of the draft genome sequence of foxtail millet paved the way for several studies that identified genes responsible for molecular and physiological processes. These genes may play a pivotal role in the growth and development of the crop and protect the plant from various environmental stresses. Characterization of genes also threw light on the regulation of few genes. The foxtail millet specific genes might also play a major function in making it a climate-resilient crop. In foxtail millet, 586 genes were observed by Zhang et al. (2012) associated with "response to water" that could be putative candidates in studying dehydration and drought stress machinery. Many genes were identified and characterized at a genome-wide level that showed differential expression when exposed to various abiotic stress viz. *NAC* (Puranik et al. 2013), *AP2/ERF* (Lata et al. 2014), *WD40* (Mishra et al. 2014), *MYB* (Muthamilarasan et al. 2014b), C_2H_2 type *zinc finger* (Muthamilarasan et al. 2014a), Nuclear factor *Y* (Feng et al. 2015), *WRKY* (Muthamilarasan et al. 2015), *RDR*, *AGO*, and *DCL* (Yadav et al. 2015), *14-3-3 proteins* (Kumar et al. 2015), *Heat shock protein* (Singh et al. 2016), *autophagy associated protein* (Li et al. 2016), *SET* (Yadav et al. 2016a, b), *DOF* (Zhang et al. 2017), *HD-Zip* (Chai et al. 2018), *CDPK* genes (Yu et al. 2018), *LIM* genes (Yang et al. 2019) and *C_4 photosynthetic genes* (Muthamilarasan et al. 2020). Regulation of root development by SiMYB3 showed auxin biosynthesis regulation in low nitrogen conditions (Ge et al. 2019).

7.6.4 Structural and Functional Genomic Resources Developed

With the foxtail millet genome sequence release, there was a sudden increase in the structural and functional genomic resources. After scanning the plant's genome, 28,342 microsatellite motifs were identified with an average coverage of 69 microsatellites/Mb of foxtail millet genome sequence. Out of 28,342 microsatellites, primer pairs for 21,294 were developed, and a physical map was constructed, and non-uniform distribution was observed. Chromosome 9 showed a maximum density of 46.4 per Mb and the highest average marker frequency of about 17%. Whereas chromosome number 8 showed a minimum density of about 30/Mb and the lowest average marker frequency of about 8%. The physical gap size between

the SSR markers was seen to be 24 kb. Forty percent polymorphism was seen in these markers, with 89% cross-transferability among other bioenergy grasses, millets, and cereals. Guinea grass showed a maximum percentage in transferability, with an average of 98.2% and wheat, with a minimum of 71.2% (Pandey et al. 2013). In foxtail millet, 24,828 unigenes were generated by assembling 66,027 ESTs reported in NCBI dbEST to develop 534 eSSRs (Kumari et al. 2013). The development of 5,123 ILP markers in foxtail millet was done by using EST database. These ILP markers could be used in marker-assisted breeding, comparative genome mapping, evolutionary studies, generating high-density genetic linkage maps, and genes and QTL mapping for beneficial agronomic traits (Muthamilarasan et al. 2014a; Muthamilarasan and Prasad 2015). Around 176 miRNA-based markers were developed by analyzing the genome-wide miRNAs reported in the foxtail millet and its related species and showed about 55% polymorphism and about 70% cross-transferability. These markers also showed good reproducibility, high stability, and efficiency (Yadav et al. 2014). The plant genome contains a substantial amount of transposable elements and is uniformly distributed in the genome, making it easier to develop TE-based markers. In foxtail millet, 30,706 TEs were found that were further divided into two classes with 6314 class I retrotransposons and 24,392 class II DNA retrotransposons. Repeat junction markers were developed (20,278), grouped in six types namely RMAP (57), IRAP (3,239), RJM (4,451), RBIP (4,801), ISBP (7,401) and RJJM (329) (Yadav et al. 2015).

The available genome sequence and annotation have eased identifying and studying gene and gene families across the foxtail millet genome using computational methods. This also helps in learning the role of the genes in plant growth and their fight against stress. For breeding in foxtail millet, allele-specific markers were developed from the *DREB2* locus that contained a dehydration tolerance linked SNP (Lata et al. 2013). NAC TFs coding 147 genes were seen in foxtail millet, out of these 50 were used for analyzing the differential gene expression against hormone and stress conditions and reported that *SiNAC128* could be used in stress associated research (Puranik et al. 2013) Genome-wide analysis of several other transcription factors were performed to find genes encoding them, like, 110 genes encoding WRKY, 171 genes encoding AP2/ERF, 124 genes encoding C2H2 zinc finger and 209 genes encoding MYB (Lata et al. 2013; Muthamilarasan et al. 2014b, 2015). RNA silencing-related genes were studied by Yadav et al. (2015) and found putative candidates like *SiDCL06*, *SiRDR07,* and *SiAG008*. A study on ADP-ribosylation factors of rice and foxtail millet were reported to have 23 ARF proteins in rice and 25 in foxtail millet. The presence of *cis*-regulatory elements in their promoter might have a role in the regulation of stress and could be studied further.

7.6.5 Application of Structural and Functional Genomics in Genomics-Assisted Breeding

Structural and functional genomics of foxtail millet is being studied extensively to establish genetic and genomic resources that could further help know the physiological and molecular basis of tolerance to several stress factors like heat, drought, and salinity. The genomics data helps develop molecular markers, QTLs, and platforms for genotyping, which can be used in genomics-assisted breeding (GAB) for the rapid development of elite varieties of foxtail millet. Stress-responsive genetic determinants, viz. alleles, genes, and QTLs in contrasting cultivars, are identified, followed by NGS-based GAB to provide stress tolerance. Prerequisites of GAB is to perform association mapping, QTL mapping as well as recurrent selection screening.

7.7 Gene Editing Strategies Developed in Foxtail Millet

The efficiency and precision of the gene-editing tools help in the rapid development of elite varieties of crops. The proven and most effective tools of genome editing are zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), programmed homing nuclease (Meganucleases), transposons, recombinant Adeno-associated virus (rAAV), and CRISPR/Cas9. CRISPR/Cas9, owing to its simple, efficient, and robust characteristic, has become very popular. Nevertheless, optimization in the construction of vector, tissue culture, gene expression, and transformation protocol are prerequisites before applying it in a new crop (Yin et al. 2017). CRISPR/Cas9 is also capable of multiplexing. In foxtail millet, CRISPR/Cas9 has been applied to mutate the PDS gene via protoplast transfection wherein the transfection efficiency was found to be 51%, and mutagenesis efficiency was 10.2% (Lin et al. 2018). In 2019, Huang et al. used the CRISPR/Cas9 strategy to knockout the *SvLES1* gene from *S. viridis* (green foxtail, wild ancestor of foxtail millet ; Huang et al. 2019). Domesticated foxtail millet has an insertion of a retrotransposon in the gene, *Less Shattering 1,* responsible for seed shattering in *S. viridis*. In 2020, Weiss' group has developed and optimized protoplast-based multiplexed CRISPR/Cas9_Trex2 tool for genome editing in green foxtail (Weiss et al. 2020). In the study, they have targeted and knocked out *Drm1a* and *Drm1b* genes that are highly linked (domain rearranged methylase). In CRISPR/Cas9, still an efficient delivery system is needed. The genome editing of foxtail millet is still in its infancy. Nevertheless, multiple types of research (as mentioned above) are going on to develop and optimize genome editing protocols in foxtail millet. More research is done in the wild ancestor of foxtail millet, i.e., *S. viridis,* and hence an efficient CRISPR/Cas9 protocol is still lacking in *S. italic*a.

7.8 Achievements of Transgenics

In foxtail millet, the transgenics were developed using the biolistic or *Agrobacterium tumefaciens* mediated methods. Gene transfer into immature inflorescence derived embryogenic callus was performed using biolistic methods (Diao et al. 1999). In another study, the bombardment of foxtail millet explants from pollen, and inflorescence was done to develop transgenic, but the efficiency was abysmal (Dong and Duan 1999, 2000). Later, embryogenic calli derived from floret was bombarded to generate overexpression as well as RNAi lines of *SiPf40*. The study shed light on auxin homeostasis and suggested that *SiPf40* played a role in tillering (Liu et al. 2009). (Liu et al. 2005) were the first to report *Agrobacterium*-mediated transformation in foxtail millet with 6.6% efficiency. A modified version of Liu et al. protocol was used for transformation using *Agrobacterium* in calli derived from panicle and immature inflorescence (Qin et al. 2008; Wang et al. 2011). This modified version was exploited by Wang et al. to overexpress *SiLEA14* (LEA proteins' homolog; LEA: late embryogenesis abundant), the transgenic lines displayed increased tolerance against salt and drought stress (Wang et al. 2014). *SiARDP* (ABA-responsive DRE-binding protein: ARDB), along with *SiASR4* uses the ABA-dependent pathway in transgenic foxtail millet to provide drought tolerance (Li et al. 2014, 2017). In 2016, Pan et al. developed *SiLTP* (LTP: Lipid transfer protein) overexpressed and RNAi lines of foxtail millet and elucidated the function of *SiLTP* against salt and drought stress. For conferring abiotic stress tolerance following the ABA-dependent signaling pathway, *SiLTP* could be a probable candidate for *SiARDP*. An efficient transformation protocol is a prerequisite to developing transgenics, but due to genetic transformation recalcitrance in foxtail millet, not many transgenics could be developed. In foxtail millet, Santos et al. (2020) has developed an *Agrobacterium*-mediated transformation protocol with an average efficiency of 19% using seed as explant. Sood et al. (2020) has developed an efficient protocol for gene expression using *Agrobacterium* transformation in foxtail millet seeds with a transformation efficiency of 27%. Recently, Yang et al. (2020) have developed a miniature mutant of foxtail millet named Xiaomi with a tiny life cycle like Arabidopsis. An efficient transformation protocol has been developed for it and the availability of transcriptomics and genomics resources to ease research in foxtail millet. Xiaomi could act as a model to study the molecular functions of C_4 plants.

7.9 Brief Account on the Role of Bioinformatics as a Tool

7.9.1 Gene and Genome Databases

It was the Joint Genome Institute (JGI) that, for the first time, introduced the foxtail millet genome sequence in Phytozome (https://phytozome.jgi.doe.gov/pz/portal.

html) wherein the genome assembly of foxtail millet genome was around 405.7 Mb present across 336 scaffolds wherein, with approximately 1.2% gap, 400.9 Mb was present across 6791 contigs representation of around 90 data is done in nine pseudomolecule. Protein coding transcripts were present in 34,584 loci, and also 43,001 protein-coding transcripts are reported (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Sitalica). Zhang et al. (2012) hosted the foxtail millet sequence in the Foxtail millet database (http://foxtailmilletmillet.genomics.org.cn) to avail the genome sequence to the researchers. PlantGDB also hosted the foxtail millet sequence (http://www.plantgdb.org/SiGDB/). KEGG database (https://www.genome.jp/kegg/) was used to map the proteins of foxtail millet. Gramene database is an open resource with integrated data containing the genome data of foxtail millet (http://www.gramene.org/Setaria_italica/Info/Index) (Tello Ruiz et al. 2016). The databases are an open resource and contain tools that could help in sequence assembly, bowser for datasets, special datasets, and more. The Foxtail millet Marker Database (FmMDb) was created in 2013 as an open-source where the large-scale datasets of markers could be retrieved, visualized, and managed in order to develop elite cultivars of the crop (http://www.nipgr.res.in/foxtailmillet.html). Several other browsers like Ensembl and UCSC Genome Browser can also be used for gene and genome search.

7.9.2 Comparative Genome Databases

Databases like Gramene, PlantGDB, Phytozome v12.1, and NCBI are used for comparative genome analysis of foxtail millet. These databases contain the integrated and updated data of the crop species. They also provide several tools that could ease the data search process wherein data can be downloaded, analyzed, or developed. They include various search functions like BLAST, pBLAST, gene viewer, and so on. Gradually with time, many application-based databases were created. *Setaria italica* functional genome database (SIFGD) is a platform created in lieu to integrate sequences of the genome, transcript, protein, miRNA, and RNA of foxtail millet from different public databases like BGI, NCBI, Phytozome, and so on (http://structuralbiology.cau.edu.cn/SIFGD/index.html). MiRNA database for foxtail millet (FmMiRNADb) contains the data for 355 miRNAs of foxtail millet and 123 molecular markers based on miRNA that would aid in molecular breeding also genotyping of cereals and millets. Foxtail millet transposable elements-based marker database (FmTEMDb) was developed with TEs data of approximately 30,000 foxtail millet with markers of six types that could be applied for a large-scale genotyping (http://59.163.192.83/ltrdb/index.html).

7.9.3 Gene Expression Databases

The Foxtail millet Transcription Factor Database (FmTFDb) was created in the year 2014. It was an open-access platform and contained 2295 transcription factors across 55 families. The transcription factor related information like sequence, phylogeny, and gene ontology could be looked into using several tools like BLAST search and set of annotation query interfaces (http://59.163.192.91/FmTFDb/index.html). NCBI (https://www.ncbi.nlm.nih.gov/guide/genes-expression/) contains data of foxtail millet gene expression along with tools to analyze the gene expression data. Also, Phytozome can be used to study the gene expression data of foxtail millet (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Sitalica). These databases provide functional annotation of several tissue-specific expression data and other corresponding information.

7.9.4 Protein or Metabolome Databases

To map the proteins of foxtail millet, SwissProt/TrEMBL (https://www.uniprot.org/statistics/TrEMBL) is extensively used as it gives easy access to the sequence of the protein and their annotation. It contains several tools like sequence BLAST, alignment, retrieval and ID mapping, and peptide search. Protein function analysis could be done by InterPro (https://www.ebi.ac.uk/interpro/). It could help predict protein domains and classify them in protein families using predictive signature models available from other member databases. Plant metabolic pathway databases (PMN) help generate a framework to mingle different plant metabolism data sources. These encompass the metabolic pathways of various plant species and corresponding enzymes, genes, and substrate (https://pmn.plantcyc.org/SETARIA). The plant metabolome database (PMDB) contains the data of plant metabolites and small molecules that are functionally and structurally annotated. For quick access, the web interface has several tools where a query can be searched, followed by data retrieval and analysis.

7.9.5 Integration of Different Data

Biological databases have turned out to be one of the crucial resources for researchers worldwide and are used daily. All the databases mentioned above may differ in functions, but they share a similar framework and show the interests and expertise of people who manage those databases. Each database has its own specialty and role, wherein it becomes difficult to answer questions related to other databases. The integration of these databases can hamper the information resource as the process will require several unwanted compromises. For instance,

maintaining the exact name of the biological samples may vary across the databases. The task of continuously updating the integrated databases would also be problematic as the biological databases always change. A single database for all the queries might make our work more comfortable, but it does not seem possible shortly. Many databases have formed a consortium so that they can exchange the information by cross-database search of queries.

7.10 Conclusions and Future Perspectives

Compared to other major cereal crops, foxtail millet is higher in nutritional compounds and can be grown in adverse climatic conditions. Lodging is one of the significant drawbacks in foxtail millet yield loss and poor grain quality, but cultivars like Longgu 28 and Nenxian 13 developed by China are lodging resistant (Dwivedi et al. 2012). Waterlogging is also one of the constraints in foxtail millet cultivation. Lugu No. 7, a foxtail millet cultivar resistant to waterlogging, was developed by Chen and Qi (1993). Studies are conducted to identify QTLs associated with climate resilient traits (Fig. 7.1).

Cereal grain cultivation is affected by the changing climatic conditions and decreasing fertility and area of agricultural land. The population worldwide is increasing, and so is food scarcity, and therefore, food production across the globe calls for an urgent solution. Speeding up crop improvement through genomic assisted breeding could help identify abiotic stress-responsive genes with better

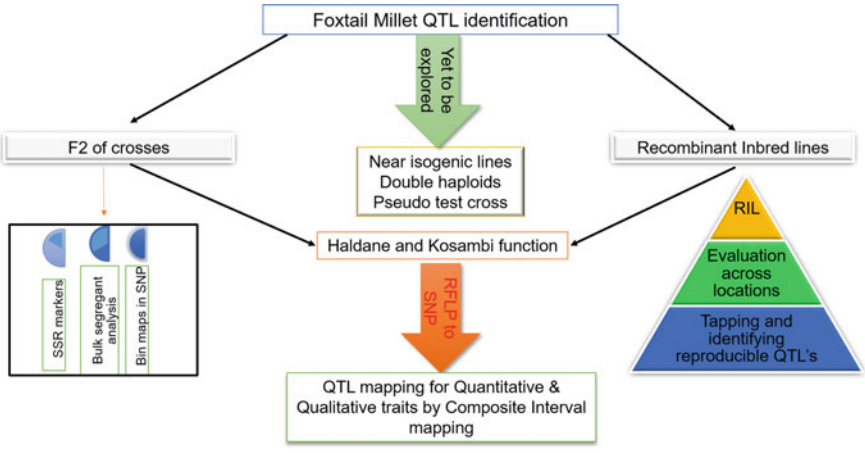

Fig. 7.1 Strategies involved in identification of quantitative trait loci controlling climate-resilient traits in foxtail millet

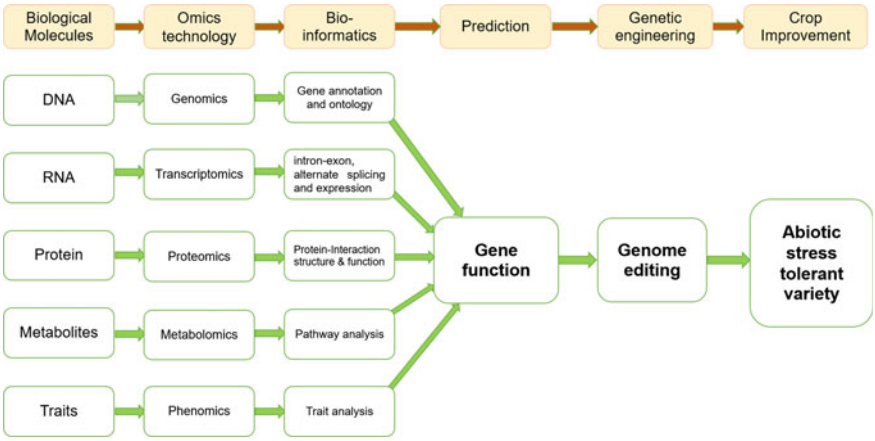

Fig. 7.2 Flowchart showing the strategies involving multi-omics tools to develop abiotic stress tolerant cultivars of foxtail millet

agronomic characters. Integration of data from multi-omics technology (genomics, transcriptomics, proteomics and metabolomics), statistical analysis, and computational biology in breeding would fasten up the crop improvement process and produce elite variety that can withstand adverse weather and give higher yield (Fig. 7.2). After the release of the reference genome, molecular maps were constructed for the tolerant and susceptible cultivars using QTLs, molecular markers, and many other candidate genes. These maps are brought into use in MAS. Speed breeding strategy can be integrated with breeding approaches like genomic assisted breeding and breeding with haplotypes to fast track the release of elite varieties. Until now, breeding was the sole source of foxtail millet improvement. With the availability of the reference genome sequence, genome editing tools, and the efficient transformation protocols discussed in the previous section can also be implied for rapid gene discovery manipulation of traits with better agronomic characteristics. These researches are majorly limited to multinational companies, and hence collaboration and coordination of the public sector are required to accelerate the release of elite varieties.

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References

Austin D (2006) Fox-tail millets (Setaria: Poaceae)—abandoned food in two hemispheres. Econ Bot 60:143–158

Banu H, Gowda J, Gowda MC (2018) Characterization and identification of DUS traits in reference set of foxtail millet germplasm (*Setaria italica* (L.) Beauv). Intl J Curr Microb Appl Sci 7(12):175–183

Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J et al (2012) Reference genome sequence of the model plant Setaria. Nat Biotechnol 30(6):555–561

Brunda SM, Kamatar MY, Hundekar R, Naveenkumar KL (2015) Studies on correlation and path analysis in foxtail millet [*Setaria italica* (L.) P. B.]. Intl J Appl Agric Hortic Sci 6(5):966–969

Ceccarelli S, Grando S (1996) Drought as a challenge for the plant breeder. Plant Growth Regul 20:149–155. https://doi.org/10.1007/BF00024011

Chai W, Si W, Ji W, Qin Q, Zhao M et al (2018) Genome-wide investigation and expression profiling of HD-Zip transcription factors in foxtail millet (*Setaria italica* L.). BioMed Res Intl 2018(8457614):18. https://doi.org/10.1155/2018/8457614

Chander S, Bhat KV, Kumari R, Sen S, Gaikwad AB et al (2017) Analysis of spatial distribution of genetic diversity and validation of Indian foxtail millet core collection. Physiol Biol Plants 23(3):663–673

Chen J, Qi Y (1993) Recent developments in foxtail millet cultivation and research in China. In: Riley KW, Gupta SC, Seetharam A, Mushonga JN (eds) Advances in small millets. Oxford and IBH Publishing Co, New Delhi, India, pp 101–107

Deaton A, Dreze J (2009) Food and nutrition in India: facts and interpretations. Available at http://www.princeton.edu/deaton/downloads/FoodandNutritioninIndiaFactsandInterpretations.pdf

Devos KM, Wang ZM, Beales CJ, Sasaki T, Gale MD (1998) Comparative genetic maps of foxtail millet (Setaria Italica) and rice (Oryza sativa). Theor Appl Genet 96:63–68

Diao X, Jia G (2017) Foxtail millet breeding in China. In: Doust A, Diao X (eds) Genetics and genomics of setaria. Springer International Publishing, Cham, Switzerland, pp 93–113

Diao X, Chen Z, Duan S, Liu Y, Zhao L et al (1999) Factors influencing foxtail millet embryogenic calli transformation by particle bombardment. Acta Agric Boreali Sin 14(3):31–36

Diao X, Schnable J, Bennetzen JL, Li J (2014) Initiation of Setaria as a model plant. Front Agric Sci Eng 1:16. https://doi.org/10.15302/J-FASE-2014011

Dong Y, Duan S (1999) Establishment of embryogenic cell suspension culture and plant regeneration of millet and gene transfer. J Basic Sci Eng 7(1):34–40

Dong Y, Duan S (2000) Production of transgenic millet plants via particle bombardment. Acta Bot Boreal-Occident Sin 20(2):175–178

Doust AN, Devos KM, Gadberry MD, Gale MD, Kellogg EAl (2004) Genetic control of branching in foxtail millet. Proc Natl Acad Sci USA 101:9045–9050

Doust AN, Devos KM, Gadberry MD, Gale MD, Kellogg EA (2005) The genetic basis for inflorescence variation between foxtail and green millet (Poaceae). Genetics 169(3):1659–1672

Dvořáková Z, Čepková PH, Janovská D, Viehmannová I, Svobodová E et al (2015) Comparative analysis of genetic diversity of 8 millet genera revealed by ISSR markers. Emir J Food Agri 617–628

Dwivedi S, Upadhyaya H, Senthilvel S, Hash C, Fukunaga K et al (2012) Millets: genetic and genomic resources. In: Janick J (ed) Plant breeding reviews, vol 35. Wiley, USA, pp 247–375

Fang X, Dong K, Wang X, Liu T, He J et al (2016) A high density genetic map and QTL for agronomic and yield traits in Foxtail millet [*Setaria italica* (L) P Beauv]. BMC Genom 17 (1):336

Feng ZJ, He GH, Zheng WJ, Lu PP, Chen M et al (2015) Foxtail Millet NF-Y Families: Genome-wide survey and evolution analyses identified two functional genes important in abiotic stresses. Front Plant Sci 6:1142

Fukunaga K, Domon E, Kawase M (1997) Ribosomal DNA variation in foxtail millet, *Setaria italica* (L) P Beauv, and a survey of variation from Europe and Asia. Theor Appl Genet 95(5–6):751–756

Fukunaga K, Wang Z, Kato K, Kawase M (2002) Geographical variation of nucleargenome RFLPs and genetic differentiation in foxtail millet, *Setaria italica* (L) P Beauv. Genet Resour Crop Evol 49(1):95–101

Fukunaga K, Kato K (2003) Mitochondrial DNA variation in foxtail millet, Setaria italica (L.) P. Beauv. Euphytica 129:7–13

Ge L, Dou Y, Li M, Qu P, He Z et al (2019) SiMYB3 in foxtail millet (*Setaria italica*) confers tolerance to low-nitrogen stress by regulating root growth in transgenic plants. Intl J Mol Sci 20. https://doi.org/10.3390/ijms20225741

Hirschi KD (2009) Nutrient biofortification of food crops. Annu Rev Nutr 29:401–421

Huang P, Mamidi S, Healey A, Grimwood J, Jenkins J et al (2019) The Setaria viridis genome and diversity panel enables discovery of a novel domestication gene.bioRxiv 2019:744557

Huang Z, Zhao L, Chen D, Liang M, Liu Z et al (2013) Salt stress encourages proline accumulation by regulating proline biosynthesis and degradation in Jerusalem Artichoke Plantlets. PLoS ONE 8(4):e62085

Jaiswal V, Gupta S, Gahlaut V, Muthamilarasan M, Bandyopadhyay T et al (2019) Genome-wide association study of major agronomic traits in foxtail millet (*Setaria italica* L) using ddRAD sequencing. Sci Rep 9(1):1–11

Jia X, Zhang Z, Liu Y, Zhang C, Shi Y et al (2009) Development and genetic mapping of SSR markers in foxtail millet [*Setaria italica* (L) P Beauv]. Theor Appl Genet 118(4):821–829

Jia X, Zhang Z, Liu Y, Zhang C, Shi Y, et al (2009) Development and genetic mapping of SSR markers in foxtail millet [Setaria italica (L) P Beauv]. TheorApplied Genet 118(4):821-829

Kole C, Muthamilarasan M, Henry R, Edwards D, Sharma R et al (2015) Application of genomics-assisted breeding for generation of climate resilient crops: progress and prospects. Front Plant Sci 6:563

Kumar K, Muthamilarasan M, Bonthala VS, Roy R, Prasad M (2015) Unraveling 14-3-3 proteins in C4 panicoids with emphasis on model plant Setaria italica reveals phosphorylation-dependent subcellular localization of RS splicing factor. PLoS ONE 10:

Kumari K, Muthamilarasan M, Misra G, Gupta S, Subramanian A et al (2013) Development of eSSR-markers in Setaria italica and their applicability in studying genetic diversity, cross-transferability and comparative mapping in millet and non-millet species. PLoS ONE 8(6):

Lata C, Gupta S, Prasad M (2013) Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33:328–343

Lata C, Mishra AK, Muthamilarasan M, Bonthala VS, Khan Y et al (2014) Genome-wide investigation and expression profiling of AP2/ERF transcription factor superfamily in foxtail millet (*Setaria italica* L). PLoS ONE 9(11):

Liu X, Tang S, Jia G, Schnable JC, Su H, et al (2016) The C-terminal motif of SiAGO1b is required for the regulation of growth, development and stress responsesin foxtail millet (Setaria italica (L.) P. Beauv). J Exp Bot 67:3237–3249

Li Y-M (1997) Breeding for foxtail millet drought tolerant cultivars (in Chinese). In: Li Y (ed) Foxtail millet breeding. Chinese Agriculture Press, Beijing, China, pp 421–446

Li Y, Wu SZ (1996) Traditional maintenance and multiplication of foxtail millet (Setaria italica (L.) P. Beauv) landraces in China. Euphytica 87:33–38

Li C, Yue J, Wu X, Xu C, Yu J (2014) ABA-responsive DRE-binding protein gene from *Setaria italica*, SiARDP, the target gene of SiAREB, plays a critical role under drought stress. J Exp Bot 65:5415–5427

Li W, Chen M, Wang E, Hu L, Hawkesford MJ et al (2016) Genome-wide analysis of autophagy-associated genes in foxtail millet (*Setaria italica* L.) and characterization of the function of SiATG8a in conferring tolerance to nitrogen starvation in rice. BMC Genomics 17:797

Li J, Dong Y, Li C, Pan Y, Yu J (2017) SiASR4, the target gene of SiARDP from *Setaria italica*, improves abiotic stress adaption in plants. Front Plant Sci 7:2053. https://doi.org/10.3389/fpls.2016.02053

Lin CS, Hsu CT, Yang LH, Lee LY, Fu JY et al (2018) Application of protoplast technology to CRISPR/Cas9 mutagenesis: from single-cell mutation detection to mutant plant regeneration. Plant Biotechnol J 16:1295–1310. https://doi.org/10.1111/pbi.12870

Liu Y, Yu J, Zhao Q, Zhu D, Ao G (2005) Genetic transformation of millet (*Setaria italica*) by *Agrobacterium*-mediated. Chin J Agric Biotechnol 13:32–37

Liu Y, Feng X, Xu Y, Yu J, Ao G et al (2009) Overexpression of millet ZIP-like gene (SiPf40) affects lateral bud outgrowth in tobacco and millet. Plant Physiol Biochem 47:1051–1060

Liu Z, Bai G, Zhang D, Zhu C, Xia X et al (2011) Genetic diversity and population structure of elite foxtail millet [*Setaria italica* (L) P Beauv] germplasm in China. Crop Sci 51(4):1655–1663

Liu T, He J, Dong K, Wang X, Wenwen W et al (2020) QTL mapping of yield component traits on bin map generated from resequencing a RIL population of foxtail millet (*Setaria italica*). BMC Genom 21(1):141

Lu H, Zhang J, Liu KB, Wu N, Li Y et al (2009) Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. Proc Natl Acad Sci USA 106:7367–7372

Mishra AK, Muthamilarasan M, Khan Y, Parida SK, Prasad M et al (2014) Genome-wide investigation and expression analyses of WD40 protein family in the model plant foxtail millet (*Setaria italica* L). PLoS ONE 9(1):

Muthamilarasan M, Prasad M (2015) Advances in *Setaria* genomics for genetic improvement of cereals and bioenergy grasses. Theor Appl Genet 128:1–14

Muthamilarasan M, Prasad M (2017) Genetic determinants of drought stress tolerance in *Setaria*. In: Doust A, Diao X (eds) Genetics and genomics of *setaria*. Springer, Berlin, pp 267–289

Muthamilarasan M, Bonthala VS, Mishra AK, Khandelwal R, Khan Y et al (2014a) C2H2-type of zinc finger transcription factors in foxtail millet define response to abiotic stresses. Funct Integr Genom 14:531–543

Muthamilarasan M, Khandelwal R, Yadav CB, Bonthala VS, Khan Y et al (2014b) Identification and molecular characterization of MYB transcription factor superfamily in C4 model plant foxtail millet (*Setaria italica* L.). PLoS One 9:e109920

Muthamilarasan M, Venkata Suresh B, Pandey G, Kumari K, Parida S et al (2014c) Development of 5123 intron-length polymorphic markers for large-scale genotyping applications in foxtail millet. DNA Res 21:41–52

Muthamilarasan M, Bonthala VS, Khandelwal R, Jaishankar J, Shweta S et al (2015) Global analysis of WRKY transcription factor superfamily in *Setaria* identifies potential candidates involved in abiotic stress signaling. Front Plant Sci 6:910

Muthamilarasan M, Dhaka A, Yadav R, Prasad M (2016) Exploration of millet models for developing nutrient rich graminaceous crops. Plant Sci 242:89–97

Muthamilarasan M, Singh RK, Suresh BV, Rana S, Dulani P et al (2020) Genomic dissection and expression analysis of stress-responsive genes in C4 panicoid models, *Setaria italica* and *Setaria viridis*. J Biotechnol 318:57–67. https://doi.org/10.1016/j.jbiotec.2020.05.007

Nandini C, Sujata B, Tippeswamy V (2018) Characterization of foxtail millet (*Sateria italica* (L) Beauv) germplasm for qualitative and quantitative traits to enhance its utilization. Acad J Agric Res 6(5):121–129

Ni X, Xia Q, Zhang H, Cheng S, Li H et al (2016) Gene mapping of nine agronomic traits and genome assembly by resequencing a foxtail millet RIL population bioRxiv: 069625

Ni X, Xia Q, Zhang H, Cheng S, Li H et al (2017) Updated foxtail millet genome assembly and gene mapping of nine key agronomic traits by resequencing a RIL population. GigaScience 6 (2):giw005

Nadeem F, Ahmad Z, Wang R, Han J, Shen, Q, et al (2018) Foxtail Millet [Setaria italica (L.) Beauv.] Grown under low nitrogen shows a smaller root system, enhanced biomass accumulation, and nitrate transporter expression. Front Plant Sci 9:205

Nirmalakumari A, Vetriventhan M (2010) Characterization of foxtail millet germplasm collections for yield contributing traits. Elect J Plant Breed 1(2):140–147

Odonkor S, Choi S, Chakraborty D, Bello LM, Wang X et al (2018) QTL mapping combined with comparative analyses identified candidate genes for reduced shattering in *Setaria italica*. Front Plant Sci 9:918

Pan Y, Li J, Jiao L, Li C, Zhu D et al (2016) A non-specific Setaria italica lipid transfer protein gene plays a critical role under abiotic stress. Front Plant Sci 7:1752. https://doi.org/10.3389/fpls.2016.01752

Pandey G, Misra G, Kumari K, Gupta S, Parida SK et al (2013) Genome-wide development and use of microsatellite markers for large–scale genotyping applications in foxtail millet [*Setaria italica* (L.)]. DNA Res 20:197–207

Pavani A, Ratna Babu D, Kumar V, Ramesh D (2019) Characterisation of quality traits in foxtail millet germplasm (*Setaria italica* (L) Beau). Intl J Chem Studies 7(6):769–772

Pramitha JL, Joel AJ, Srinivas S, Sreeja R, Hossain R et al (2020) Enumerating the phytic acid content in maize germplasm and formulation of reference set to enhance the breeding for low phytic acid. Physiol Mol Biol Plants 26(2):353–365

Puranik S, Sahu PP, Mandal SN, Suresh VB, Parida SK et al (2013) Comprehensive genome-wide survey, genomic constitution and expression profiling of the NAC transcription factor family in Foxtail Millet (*Setaria italica* L.). PLoS One 8:e64594

Qie L, Jia G, Zhang W, Schnable J, Shang Z et al (2014) Mapping of quantitative trait locus (QTLs) that contribute to germination and early seedling drought tolerance in the interspecific cross *Setaria italica Setaria viridis*. PLoS ONE 9(7): https://doi.org/10.1371/journal.pone.0101868

Qin F, Zhao Q, Ao G, Yu JJ (2008) Co-suppression of Si401 a maize pollen specific Zm401 homologous gene, results in aberrant anther development in foxtail millet. Euphytica 163 (1):103–111

Radha BN, Channakeshava BC, Bhanuprakash K (2014) Biochemical landmarks for Identification of foxtail millet genotypes. Intl J Appl 9(1):9–26

Reddy VG, Upadhyaya HD, Gowda CLL (2006) Current status of sorghum genetic resources at ICRISAT: their sharing and impacts. Intl Sorghum Millets Newsl 47:9–13

Santos CM, Romeiro D, Silva JP, Basso MF, Molinari HBC et al (2020) An improved protocol for efficient transformation and regeneration of *Setaria italica*. Plant Cell Rep 39:501–510. https://doi.org/10.1007/s00299-019-02505-y

Sato K, Mukainari Y, Naito K, Fukunaga K, Mukainari Y et al (2013) Construction of a foxtail millet linkage map and mapping of spikelet-tipped bristles1 (stb1) by using transposon display markers and simple sequence repeat markers with genome sequence information. Mol Breed 31 (3):675–684

Schontz D, Rether B (1998) Genetic variability in foxtail millet, Setaria italica (L) P Beauv-RFLP using a heterologous rDNA probe. Plant Breed 117(3):231–234

Singh RK, Jaishankar J, Muthamilarasan M et al (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

Sood P, Singh RK, Prasad M (2020) An efficient Agrobacterium-mediated genetic transformation method for foxtail millet (*Setaria italica* L.). Plant Cell Rep 39:511–525. https://doi.org/10.1007/s00299-019-02507-w

Sun J, Luu NS, Chen Z, Chen B, Cui X et al (2019) Generation and characterization of a Foxtail Millet (*Setaria italica*) mutant library. Front Plant Sci 10:369

Tang C, Tang S, Zhang S, Luo M, Jia G et al (2019) SiSTL1, encoding a large subunit of ribonucleotide reductase, is crucial for plant growth, chloroplast biogenesis, and cell cycle progression in Setaria italica. J Exp Bot 70:1167–1182. https://doi.org/10.1093/jxb/ery429

Tello-Ruiz MK, Stein J, Wei S, Preece J, Olson A et al (2016) Comparative plant genomics and pathway resources. Nucleic Acids Res 2016 Jan 4;44(D1):D1133-40. https://doi.org/10.1093/nar/gkv1179. Epub 2015 Nov 8. PMID: 26553803; PMCID: PMC4702844

Upadhyaya HD, Gowda CLL, Sastry (2008) Plant genetic resources management: collection, characterization, conservation and utilization. J SAT Agric Res 6:16

Upadhyaya HD, Pundir RPS, Dwivedi SL, Gowda CLL, Reddy VG et al (2009a) Developing a mini core collection of sorghum for diversified utilization of germplasm. Crop Sci 49(5):1769–1780

Upadhyaya HD, Pundir RPS, Gowda CLL, Reddy VG, Sl Singh (2009b) Establishing a core collection of foxtail millet to enhance the utilization of germplasm of an underutilized crop. Plant Genet Resour 7(2):177–184

Veeranagamallaiah G, Sudhakar C (2017) Determination of polyamines by dansylation, benzoylation, and capillary electrophoresis. In: Plant stress tolerance, pp 313–323. Humana Press, New York, NY

Wang J, Wang Z, Du X, Yang H, Han F, et al (2017) A high-density genetic map and QTL analysis of agronomic traits in foxtail millet [Setaria italica (L) P Beauv] using RAD-seq. PLoS ONE 12(6):e0179717

Wang ZM, Devos KM, Liu CJ, Wang RQ, Gale MD (1998) Construction of RFLP-based maps of foxtail millet, *Setaria italica* (L) P Beauv. Theor Appl Genet 96(1):31–36

Wang M, Pan Y, Li C, Pan Y, Jiang X et al (2011) Culturing of immature inflorescences and *Agrobacterium*-mediated transformation of foxtail millet (*Setaria italica*). Afr J Biotechnol 10:16466–16479. https://doi.org/10.5897/ajb10.2330

Wang M, Li P, Li C, Pan Y, Jiang X et al (2014) SiLEA14, a novel atypical LEA protein, confers abiotic stress resistance in foxtail millet. BMC Plant Biol 14(1):290. https://doi.org/10.1186/s12870-014-0290-7

Wang Z, Wang J, Peng J, Du X, Jiang M et al (2019) QTL mapping for 11 agronomic traits based on a genome-wide Bin-map in a large F 2 population of foxtail millet (*Setaria italica* (L) P Beauv). Mol Breed 39(2):18

Watson A, Ghosh S, Williams MJ, Cuddy WS, Simmonds J et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4:23–29. https://doi.org/10.1038/s41477-017-0083-8

Weiss T, Wang C, Kang X, Zhao H, Gamo ME et al (2020) Optimization of multiplexed CRISPR/Cas9 system for highly efficient genome editing in *Setaria viridis*. Plant J 0–2. https://doi.org/10.1111/tpj.14949

Wen Q-F, Wang L, Wang XY (2005) The foxtail millet germplasm resources and screening and utilization of drought resistance germplasm in Shanxi (In Chinese with English abstract) J Shanxi Agric Sci 33:32–33

Willweber-kishiomoto E (1962) Interspecific relationships in the genus *setaria.* Contributions from the biological laboratory. Kyoto University 14:1–41 http://hdlhandlenet/2433/155927

Yadav CB, Muthamilarasan M, Pandey G, Khan Y, Prasad M (2014) Development of novel microRNA-based genetic markers in foxtail millet for genotyping applications in related grass species. Mol Breed. https://doi.org/10.1007/s11032-014-0137-9

Yadav CB, Muthamilarasan M, Pandey G, Khan Y, Prasad M (2015) Identification, characterization and expression profiling of Dicer-like, Argonaute and RNA dependent RNA polymerase gene families in foxtail millet. Plant Mol Biol Rep 33:43–55

Yadav CB, Muthamilarasan M, Dangi A, Shweta S, Prasad M (2016a) Comprehensive analysis of SET domain gene family in foxtail millet identifies the putative role of SiSET14 in abiotic stress tolerance. Sci Rep 6:32621

Yadav CB, Muthamilarasan M, Dangi A, Shweta S, Prasad M (2016b) Comprehensive analysis of 890 SET domain gene family in foxtail millet identifies the putative role of SiSET14 in abiotic 891 stress tolerance. Sci Rep 6:32621

Yang R, Chen M, Sun J, Yue Y, Min DH et al. (2019) Genome-wide analysis of LIM family genes in foxtail millet (*Setaria italica* L.) and characterization of the Role of SiWLIM2b in drought tolerance. Intl J Mol Sci 20:1303

Yang Z, Zhang H, Li X, Shen H, Gao J et al (2020) A mini foxtail millet with an Arabidopsis-like life cycle as a C4 model system. Nat Plants. https://doi.org/10.1038/s41477-020-0747-7

Yin K, Gao C, Qiu JL (2017) Progress and prospects in plant genome editing. Nat Plants 3:17107. https://doi.org/10.1038/nplants.2017.107 PMID: 28758991

Yoshitsu Y, Takakusagi M, Abe A, Takagi H, Uemeura A et al (2017) QTL-seq analysis identifies two genomic regions determining the heading date of foxtail millet, *Setaria italica* (L) P Beauv. Breed Sci 67(5):518–527

Yu T-F, Zhao W-Y, Fu J-D, Lei Y-W, Chen M et al (2018) Genome-wide analysis of CDPK family in foxtail millet and determination of SiCDPK24 functions in drought stress. Front Plant Sci 9:651. https://doi.org/10.3389/fpls.2018.00651

Zhang K, Fan G, Zhang X, Zhao F, Wei W, et al (2017) Identification of QTLs for 14 agronomically important traits in Setaria italica based on SNPs generatedfrom high-throughput sequencing. G3 (Bethesda) 7(5):1587–1594

Zhang HZ (1980), Study on the natural hybridization rate and the acquisition of sexual hybrids of millet. Shanxi Agric Sci 12–13

Zhang J, Liu T, Fu J, Zhu Y, Jia J et al (2007) Construction and application of EST library from *Setaria italica* in response to dehydration stress. Genomics 90:121–131

Zhang X, Xia J, Li YE, Blanca-figureoa BE, Gao XZ et al (2012) Genome-wide analysis of plant nat-siRNAs reveals insights into their distribution, biogenesis and function. Genome Biol 13:R20

Zhang S, Tang S, Tang C, Luo M, Jia G et al (2018) SiSTL2 is required for cell cycle, leaf organ development, chloroplast biogenesis, and has effects on C4 photosynthesis in *setaria italica* (L.) P, Beauv. Front Plant Sci 9:1–16. https://doi.org/10.3389/fpls.2018.01103

Chapter 8
Genomic-Assisted Breeding in Finger Millet (*Eleusine Coracana* (L.) Gaertn.) for Abiotic Stress Tolerance

T. P. Ajeesh Krishna, T. Maharajan, S. Ignacimuthu, and S. Antony Ceasar

Abstract The demand for more food and the prevalence of malnutrition are the major issues affecting people in several developing countries. Finger millet, (*Eleusine coracana* [L.] Gaertn). is a minor cereal with rich sources of nutraceuticals as compared to other regularly consumed major cereals. Finger millet is considered as a staple food for millions of poor people living in Asia and Africa. It is ranked fourth based on the economic importance among millets worldwide. Therefore, finger millet could help in strengthening both food and nutritional security in the future. However, the finger millet production is constrained by various abiotic stresses leading to a decrease in its yield and quality. In recent years, genome-assisted breeding has become an attractive and efficient strategy for crop improvement programs. It is considered as a "third-generation" tool of plant breeding. For genome-assisted breeding, the basic need is to have genomic information, trait-specific mapping of populations, and highly advanced phenomic facilities. The genomic study is involved in the development of new molecular markers and manipulation of quantitative trait loci (QTL) through marker-assisted selection (MAS) for the development of improved varieties. Therefore, the genomic information is very crucial for the finger millet improvement program. The whole-genome sequence (WGS) is available for finger millet. So, it provides the opportunity for finger millet improvement through genome-assisted breeding. In this chapter, we present the details on genomic and phenomic approaches undertaken for finger millet improvement. This chapter could help researchers in

T. P. Ajeesh Krishna · T. Maharajan · S. Antony Ceasar (✉)
Division of Plant Biotechnology, Entomology Research Institute, Loyola College, University of Madras, Chennai 600034, India

S. Ignacimuthu
Xavier Research Foundation, St Xavier's College, Palayamkottai 627002, India

S. Antony Ceasar
Division of Plant Molecular Biology and Biotechnology, Department of Biosciences, Rajagiri College of Social Sciences, Kalamassery, Kochi 683104, India

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understanding the importance and application of a genome-assisted breeding program in finger millet improvement to conserve future food security in the developing world.

Keywords Finger millet · Abiotic stress · Crop improvement · Finger millet germplasm · Genomic resources · Phenomics

8.1 Introduction

Food scarcity and malnutrition have been the major causes of death for children in many developing countries. A major challenge for food production in the coming decades will be to meet the demands of the growing population worldwide (Beddington 2010). In 2050, the world's population may increase upto 9.8 billion which will need more food (Vetriventhan et al. 2020). Therefore, demand for food is expected much before 2050 itself that will require an increase in the crop production up to 60–70% (Vetriventhan et al. 2020). The required crop production must be of good quality and quantity to prevent malnutrition around the world. Therefore, food and nutritionally important crops are very essential to face future challenges; it is also needed to increase their production on a large scale. The major cereal crops in the world are rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) which provide food security to majority of the population (Subash et al. 2011). Millets are referred as grainy cereals from a variety of small edible grasses (Singh and Raghuvanshi 2012). Millets are the most important minor cereal crops of the semi-arid zones of the world. Among the millets, finger millet (*Eleusine coracana* [L.] Gaertn.) has high nutritional properties and is superior to rice and wheat for nutrient profile (Shobana et al. 2013). Therefore, finger millet is termed as nutria-millet or nutria-cereals (Swaminaidu et al. 2015; Kumar et al. 2016; Puranik et al. 2017). Worldwide, 12% of the total millet area is under finger millet cultivation, covering more than 25 countries of Asia and Africa (Kumar et al. 2016). Hence, it is considered as a staple food for poor people living on marginal lands and with limited economic resources (Krishna et al. 2018). Finger millet may help in strengthening both food and nutritional security in developing countries.

Crop productivity is threatened by various stressful conditions associated with climate changes (Shibairo et al. 2016; Zhao et al. 2017; Ray et al. 2019). Crops normally deal with many forms of environmental stresses (Kumar 2013; Rejeb et al. 2014). Both biotic and abiotic constraints diversely affect finger millet production (Ramakrishnan et al. 2016a; Yamunarani et al. 2016; Maharajan et al. 2019). Hence, farmers across the world need to adopt newer techniques for increasing crop production on the available cultivable land. Difficulties in expanding agricultural land, improving soil conditions, facing climate change, combating world population, and other current societal changes force the researchers to get involved in crop improvement programms. Crop improvement has been one of the priority areas of research in finger millet. The genomic resources available for this crop are very

little when compared to other major cereal crops (Ceasar et al. 2018). Therefore, only a limited number of genetic and genomic studies have been conducted on the improvement of finger millet. This problem can be solved by finger millet germplasm, which has to be continuously improved by plant breeders, biotechnologists, and molecular biologists for the increased growth and yield of finger millet.

In recent years genetics and genomics studies have greatly enhanced the understanding of structural, behavioral, and functional aspects of plant genomes. The availability of the whole-genome sequence (WGS) of finger millet facilitates the researcher to identify the gene networks that are involved in controlling genetic variation for agronomically valuable traits in best breeding populations (Hittalmani et al. 2017; Hatakeyama et al. 2017). Genome-assisted tools help in the rapid identification and selection of novel beneficial genes in the germplasm. So, it provides the opportunity to identify candidate genes responsible for biotic and abiotic stress tolerance. This can help to improve finger millet productivity under any biotic and abiotic constraints through genome-assisted breeding programs.

In this chapter, we focus more on genome-assisted breeding strategies in finger millet for abiotic stress tolerance. We present the details on the importance of finger millet, abiotic constraints of finger millet production, and the role of genomic and phenomic approaches made for finger millet improvement. We briefly discuss various biotechnological approaches undertaken for improving the finger millet. This chapter will help plant biologists, molecular biologists, and plant breeders to understand the importance and application of the genome-assisted breeding program for finger millet improvement in future.

8.2 Origin and Importance of Finger Millet

The cultivated finger millet is a tetraploid ($2n = 4x = 36$; AABB); it is a self-pollinating species. It exhibits morphological similarity to both *E. indica* ($2n = 18$) and *E. Africana* ($2n = 36$). It is derived from the wild species *E. indica* and *E. tristachya* or *E. floccifolia* (Hiremath and Salimath 1992; Babu et al. 2013). The cultivated finger millet was domesticated in western Uganda and Ethiopia around 5,000 years BC before getting introduced in India, approximately 3,000 years BC. Finger millet is one of the most important small millet crops grown in large areas of the developing world especially in Africa and Asia (Ceasar et al. 2018).

Wide adaptability, better nutritional quality, promising health benefits, and high multiplication rate make finger millet an ideal crop to use as a staple food crop in developing countries (Poonia et al. 2012; Dhanalakshmi et al. 2014; Isingoma et al. 2015; Gupta et al. 2017) which is considered as an important component of food security. Additionally, it can be stored for several years without the attack of storage pests, which makes it a perfect food crop for the semi-arid zones of the world. Finger millet is a good source of nutrients including calcium (344 mg/100 g), potassium (408 mg/100 g), fats (1.4%), other minerals (2.7%), and fiber (18%) (Poonia et al. 2012; Shobana et al. 2013; Thapliyal and Singh 2015). The total

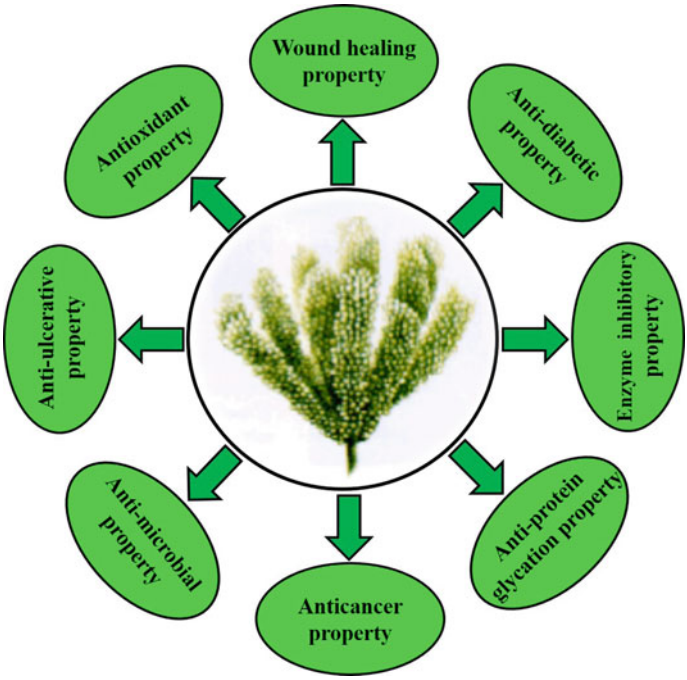

Fig. 8.1 Health benefits of finger millet in humans. The diagram illustrates various health benefits of finger millet in humans

carbohydrate content of finger millet is 72–79.5% (Joshi and Katoch 1990; Pore and Magar 1979). Also, it contains 44.7% essential amino acids (Mibithi-Mwikya et al. 2000), which are vital for human health (Fig. 8.1). Therefore, it is an important food crop for people with a low socio-economic input (Sripriya et al. 1997) and for those suffering from metabolic disorders (Mathanghi and Sudha 2012). Finger millet is considered a dual-purpose crop providing food grain and dry fodder. Finger millet straw serves as a good cattle fodder which contains up to 61% digestible nutrients (National Research Council 1996).

Nowadays, nutritional biology focuses extensively on the development of food-based nutraceuticals that are beneficial to human health. Finger millet is a crop with a rich nutraceutical properties as compared to other regularly consumed cereals. Therefore, apart from its important uses and nutritive value, the medicinal properties of finger millet are also beneficial to human health.

8.3 Abiotic Stresses in Finger Millet

Abiotic stress is another major constraint to global food security besides affecting yield and quality of produce. It severely affects the growth and yield of finger millet (Saha et al. 2016; Ceasar et al. 2018; Maharajan et al. 2019). The global finger

millet production is around 4.5 million tons per year (Onyango 2016). The yield of finger millet varies across countries and regions due to their soil types and climate (Sood et al. 2016). Low soil fertility (nutrient deficiency), soil moisture incidence (drought), and elevated soil salinity are major abiotic stresses in finger millet (Krishnamurthy et al. 2014; Maharajan et al. 2019; Mukami et al. 2020; Talwar et al. 2020). Among these, drought is the most important abiotic constraint in the finger millet producing area of the world (Ceasar et al. 2018). Drought inhibits plant growth and development, and hence productivity through disturbing various physiological and biochemical processes. Drought stress can occur at any time of the life periods of the crop like seedling stage, vegetative growth, or grain filling. Exposure to drought causes wilting and leaf rolling (Parvathi et al. 2013) and the droopy appearance of the shoots and leaves turning inward from the outside edges (Bhatt et al. 2011). Finger millet responds to drought by enhancing its anti-oxidative capacity (Bhatt et al. 2011) and induction of many drought stress-responsive genes (Parvathi et al. 2013), which contribute to the potential of finger millet being an abiotic stress tolerant crop.

Similarly, nutrient deficiency is another important issue that affects the productivity and quality of finger millet (Yamunarani et al. 2016; Ramakrishnan et al. 2017; Maharajan et al. 2019). The nutrient deficiency was observed in a wide range of soil types such as sandy and calcareous soil (Rashid and Ryan 2004; Alloway 2009). These soils show low organic carbon (C) and deficiency in nitrogen (N), phosphorus (P) and zinc (Zn), which may affect finger millet production (Safaa and Fattah 2007; Ceasar et al. 2018). Goron et al. (2015) reported that N deficiency decreased the tiller number in finger millet. P deficiency also affected the plant growth, biomass, and yield of finger millet seedlings in greenhouse conditions (Ramakrishnan et al. 2017; Maharajan et al. 2019). The Zn deficiency showed a decrease in the grain yield in finger millet (Yamunarani et al. 2016). Therefore, identification and characterization of low nutrient stress tolerant genes are very crucial for the development of improved varieties of finger millet.

Soil salinity is also an important constraint that impacts finger millet production (Krishnamurthy et al. 2014; Mukami et al. 2020), but this issue is not considered as a widespread problem like drought and nutrient deficiency. In finger millet, salinity stress reduced the water content, plant height, leaf expansion and finger length and width; it also delayed the flowering and reduced grain weight (Anjaneyulu et al. 2014). Another study showed that salinity stress reduced the finger millet growth and terminal leaf elongation rate (Rahman et al. 2014). Similarly, salinity stress showed a significant reduction in plant growth and influenced shoot and root biomass in finger millet genotype GPU-28 (Hema et al. 2014; Parvathi and Nataraja 2017). All the abiotic constraints significantly affect the finger millet production. Therefore, germplasm characterization and gene discovery are essential for developing abiotic stress tolerant finger millet for enhancing its production.

8.4 Finger Millet Germplasm

Diverse genetic resource is essential for the genetic improvement of any crop including finger millet (Upadhyaya et al. 2007). Diverse germplasm helps in the identification and characterization of valuable abiotic stress tolerant QTL/genes. Recently, many abiotic stress-tolerant genotypes were identified in the mini-core collection of finger millet germplasm (Krishnamurthy et al. 2014, 2016; Ramakrishnan et al. 2017; Krishna et al. 2020b). The finger millet germplasm consists of more than 22,799 accessions worldwide, including wild relatives, landraces, breeding/research materials, and improved varieties, etc. (Goron and Raizada 2015). The highest collection was recorded from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India which holds the 10,507 finger millet accessions. Most of these accessions are indigenous in nature with only 117 accessions of non-Indian origin. These Indian collections include 154 improved varieties, six wild relatives, and 64 breeding/research material (Mathur 2012). Moreover, a total of 5,957 finger millet accessions are preserved by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. It includes 5,665 landraces, 137 improved cultivars, 105 wild species and 50 breeding/research materials. Out of 5,957 finger millet accessions, 4,585 accessions are of non-Indian origin (Mathur 2012). The United States Department of Agriculture (USDA) at Griffin, Georgia, maintains 766 finger millet accessions, comprising of 17 wild types (Mathur 2012; Sood et al. 2016). These accessions belong to 11 countries; the accessions are from India (646), Uganda (54), Nepal (4), Kenya (3), Zaire (3), Zimbabwe (2), Ethiopia (2), Pakistan (2), South Africa (1), Tanzania (1) and Zambia (1). The other Asian and African countries are holding considerable collections of finger millet accessions including Kenya (1,902), Zimbabwe (1,158), Uganda (1,155), Nepal (877), Sri Lanka (393) and Bhutan (84) (Mathur 2012; Goron and Raizada 2015; Gupta et al. 2017). These core finger millet germplasm collections could be effectively evaluated and used in breeding programs. Therefore, germplasm collection and characterization are preliminary and crucial steps in crop improvement programs. A huge number of finger millet accessions provide the opportunity to improve the finger millet through genomic and phenomic studies in future.

8.5 Germplasm Characterization and Gene Discovery

The germplasm characterization and gene discovery are essential for finger millet improvement programs. Also, they are important link between the conservation and utilization of finger millet genetic resources. The contrasting characters of finger millet accessions for economic traits are useful in inheritance studies and developing trait-based linkage mapping populations (Dhanalakshmi et al. 2014). The variability existing in the finger millet germplasm provides opportunities for

breeders to choose specific donors for genetic improvement (Ulaganathan and Nirmalakumari 2015). It is helpful for effective selection in the segregating generations for the development of high yielding varieties. Similarly, the genome sequence information allows the development of molecular marker, construction of genetic maps, identification of genes, alleles and QTL, etc. In genomic-assisted breeding, marker-assisted selection (MAS) is very useful since it is not interfered by any environmental condition and provides accuracy during the phenotypic selection (Krishna et al. 2020a). The transcriptome sequence also provides the gene involvement of the trait of interest, which helps to develop genetically improved plants through transgenic approaches (Krishna et al. 2017). Assessment of phenomic and genomic studies may be helpful for choosing genotypes for further breeding works (Krishna et al. 2017; Sood et al. 2019). So, high-throughput phenomic and genomic data are essential for efficient finger millet crop improvement through precision breeding (Fig. 8.2).

8.5.1 Phenomics

Phenomics is a systematic way of studying the phenotypes. Phenotyping is still non-uniform, labor-intensive, and environmentally sensitive. But, it is very crucial for germplasm characterization. In recent years, new technologies are available in phenomic studies. Availability of automation, imaging, and software has helped in high-throughput phenotyping studies. It integrates and optimizes a phenotyping process in a way that makes it efficient (Sood et al. 2019). Therefore, high-throughput phenotyping system provides uniformity of data at any time. Plant functional traits such as morphological, phenological, physiological, and nutritional characters determine how plants respond to abiotic stresses. Hence, germplasm evaluation and characterization are also important for plant breeders. A large amount of variability existing in the germplasm provides an opportunity for the plant breeder to undertake further breeding activities (Ulaganathan and Nirmalakumari 2015). These days efficient and standard hybridization protocols are available in finger millet (Sood et al. 2019; Krishna et al. 2020b) which may help to accelerate the breeding programs for variety improvement.

Morphological markers/characters play an important role in germplasm characterization (Dasanayaka 2016) (Fig. 8.3). Recently, Suman et al. (2019) characterized 55 finger millet genotypes based on their agro-morphological traits under field conditions. Grain yield per plant, productive tillers per plant, days to flowering, 1,000-grain weight, days to maturity, finger number per panicle, finger length, and finger width were taken into account for the characterization of these genotypes. These agronomically important traits are contributing to the genetic divergence of the genotypes (Suman et al. 2019). Similarly, agro-morphological traits of 305 genotypes were evaluated under field condition and the genotypes such as TNEc 1242, TNEc 1872, TNEc 1747 and TNEc 2092 were found to be more variable based on principal component analysis (PCA) (Ulaganathan and

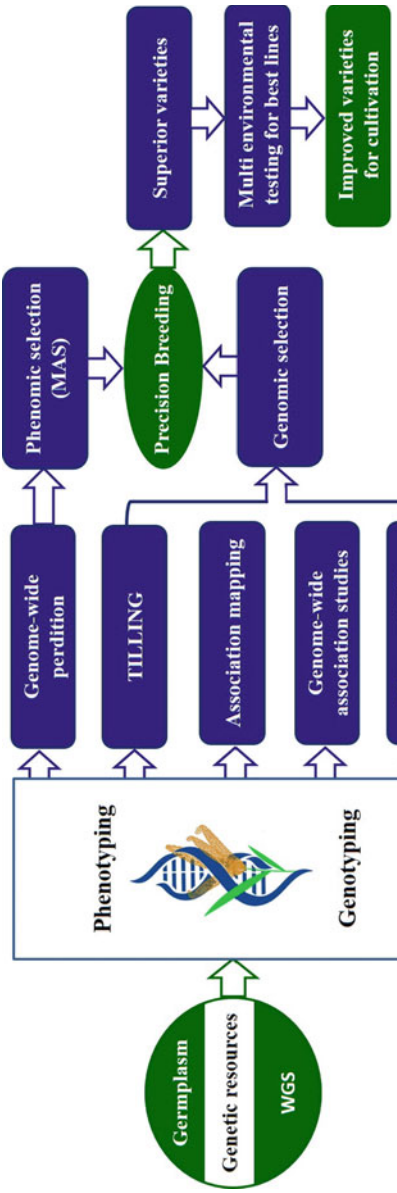

Fig. 8.2 Phenomic and genomic approaches for finger millet improvement through precision breeding. This flowchart describes the phenomic and genomic approaches in finger millet germplasm for developing improved varieties. [*Abbreviations* WGS whole genome sequence; TILLING targeting induced local lesions in genomes; QTL quantitative trait loci; MAS marker-assisted selection]

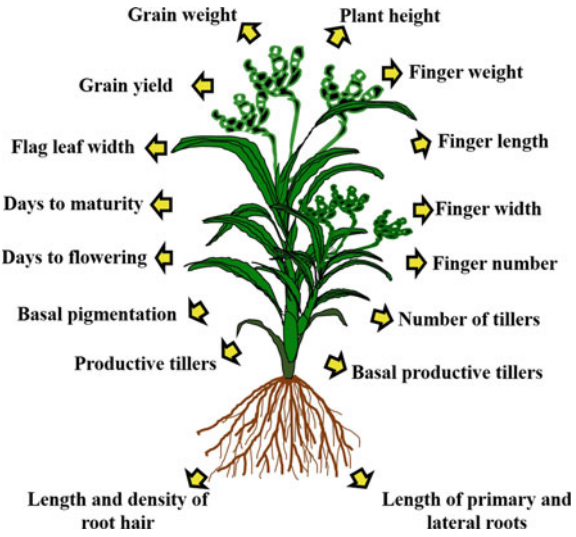

Fig. 8.3 Important agro-morphological traits for finger millet germplasm characterization. Details on agronomically important traits used for finger millet improvement are presented

Nirmalakumari 2015). Several studies have been performed in finger millet to assess their agro-morphological traits (Table 8.1). Furthermore, a total of 622 genotypes were screened to find the best and worst-performing genotypes in field conditions based on the plant height, the number of productive tillers and grain weight. The genotype IE 2322 was found to be the best and IE 6059 was the worst genotype (Dhanalakshmi et al. 2014).

So far, only a few studies have been performed on nutritional and nutrient aspects of finger millet phenotyping. Ramakrishnan et al. (2017) grew 128 genotypes of finger millet in low P condition under greenhouse and categorized these into low-P-tolerant and low-P-susceptible genotypes. The genotypes such as GPU45, IE5201, IE2871, IE7320, GPU66, HOSUR1, TCUM1, IE2034, SVK1, RAU8, VR708, and IE3391 were found to be low-P-tolerant genotypes (Ramakrishnan et al. 2017). Based on the nutritional aspects, totally 319 finger millet genotypes were used to analyze their seed Zn content. In this study, the genotypes GEC164 and GEC543 showed higher grain Zn content compared to other genotypes (Yamunarani et al. 2016). Similarly, Nirgude et al. (2014) analyzed 106 genotypes for their genetic variation in protein and calcium content in grain. Great variability in protein and calcium content were seen in these genotypes (Nirgude et al. 2014). Further, Mukami et al. (2019) investigated the biochemical and physiological responses of six finger millet genotypes (GBK043122, GBK043124, GBK043128, GBK043137, GBK043050 and GBK043094) under drought stress condition. These results showed that biochemical and physiological traits contributed to drought tolerance in GBK043137 and GBK043094 genotypes. These genotypes were less affected by drought than the other varieties as shown by significant changes in their physiological parameters (Mukami et al. 2019). These studies help in phenotypic selection for breeding purposes. The appreciable

Table 8.1 Analysis and characterization of finger millet germplasm

Number of finger millet accessions	Name of trait	Findings	Reference
10	Morphological traits	Variability of different morphological traits between the accessions	Umar and Kwon-Ndung (2014)
30	Morphological traits	The genotypes such as PRM 9003, GEC 961, GEC 268, GEC 1044, GEC 268, VL 149 and GEC 199 showed better performance	Goswami et al. (2015)
909	Morphological traits	Diverse morphological traits	Upadhyaya et al. (2007)
55	Agro-morphological traits	Great genetic diversity for morpho-agronomical traits	Suman et al. (2019)
622	Agro-morphological traits	The accessions (IE 2322 and IE 6059) are contrasting for plant height, number of productive tillers and grain yield	(Dhanalakshmi et al. 2014)
305	Agro-morphological traits	Genotypes TNEc 1242, TNEc 1872, TNEc 1747 and TNEc 2092 are found to be more variable	Ulaganathan and Nirmalakumari (2015)
190	Agro-morphological characters	Identified the important agro-morphological QTL	Babu et al. (2014a)
60	Agronomic traits	High heritability is observed in genotypes based on their agronomically important traits	Tesfaye and Mengistu (2017)
2000	Qualitative and quantitative traits	Substantial diversity is observed in all qualitative and quantitative traits	Reddy et al. (2009)
1000	Qualitative and quantitative traits	Observed different genetic diversity based on qualitative and quantitative traits	Haradari et al. (2012)
24	Quantitative traits	Identified the better performance genotypes based on quantitative traits	Dasanayaka (2016)
128	Low P stress	Identified low P tolerant genotypes such as GPU45, IE5201, IE2871, IE7320, GPU66, HOSUR1, TCUM1, IE2034, SVK1, RAU8, VR708 and IE3391	Ramakrishnan et al. (2017)
–	Low Zn stress	Identified the low-Zn-tolerant (IE 2606) and low-Zn-susceptible (PR 202) genotypes	Krishna et al. (2020b)

(continued)

Table 8.1 (continued)

Number of finger millet accessions	Name of trait	Findings	Reference
06	Drought stress	Observed physiological and biochemical responses of genotypes under drought. Genotypes GBK043137 and GBK043094 showed drought stress tolerance	Mukami et al. (2019)
38	Drought stress	Genotypic performance (drought tolerant) was observed under field conditions	Talwar et al. (2020)
103	Grain protein and calcium content	Observed a significant variation of protein and calcium content in grain	Nirgude et al. (2014)
319	Seed Zn content	The great variation for grain Zn content among genotypes. Genotypes GEC164 and GEC543 showed higher grain Zn content	Yamunarani et al. (2016)

The details such as the number of accessions, name of trait and findings are provided

variation in grain nutrient content and tolerance to abiotic stresses among finger millet genotypes offers opportunities to improve quality and productivity through breeding approaches.

8.5.2 Genomics

The genomic resources are very crucial for genome-assisted breeding and functional genomic studies. Genetic maps, molecular markers, and sequence information are some of the basic genomic resources (Bansal et al. 2014), which are required for crop improvement programs. Genome based research has reduced the time and effort involved in direct screening of germplasm grown under field and greenhouse conditions. Therefore, conventional plant breeding has gradually changed from phenotype-based to genotype-based selection (Leng et al. 2017). Genomic research is also involved in the development of new molecular markers and identification of QTL through MAS for the development of improved varieties (Maharajan et al. 2018). In general, finger millet has very little genomic resources as compared with other major cereal crops, which hampers the further improvement of this crop (Saha et al. 2016; Ceasar et al. 2018). Therefore, finger millet genomic resource is essential for its improvement.

8.5.2.1 Finger Millet Genome Sequence

Next-generation sequencing (NGS) is a most advanced technology of genome sequencing, which helps in sequencing and understanding the genome organization of many crops. Whole-genome assembly of finger millet genotype ML-365 (Hittalmani et al 2017), and PR 202 (Hatakeyama et al. 2017) was done using Illumina and SOLiD sequencing technologies, respectively. The whole genome sequencing (WGS) of finger millet genotype ML-365 showed around 45 Gb paired-end, 21 Gb mate-pair data, and 525,759 scaffolds (>200 bp) with an N50 length of 23.73 Kb and the average scaffold length of 2,275 bp (Hittalmani et al. 2017). Also, numerous genes for disease resistance (1,766), drought-responsive (2,866), and calcium transport and accumulation (330) were identified. The average DNA content (2C) and genome size of finger millet genotype ML-365 were 3.01 pg and 1,453 Mb respectively. The WGS covered approximately 82% of the total estimated genome size of finger millet (Hittalmani et al 2017). The WGS analysis revealed the presence of 85,243 genes (Hittalmani et al 2017). Similarly, finger millet genotype PR-202 genome is 1.5 Gb, and the assembled genome had 1,189 Mb covering only 78.2% genome (Hatakeyama et al. 2017) (Table 8.2). The WGS of finger millet was found to have greater colinearity with the model millet crop foxtail millet (Ceasar et al. 2018). The genome sequence of foxtail millet is well annotated, and is available in Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html). But, the finger millet genome sequence is not yet fully annotated. Hence, the well-annotated genome sequence of foxtail millet could help for comparative-genome studies. Well-annotated finger millet genome sequence is also needed for a better understanding of genome organization and high-resolution studies in the future for finger millet improvement. The details with the comparison of WGS of foxtail millet and finger millet are given in Table 8.2.

Moreover, only a few efforts were made to sequence the transcriptome of specific genotypes subjected to stresses like drought, water, moisture, and salinity (Table 8.3). So, more transcriptome sequencing of specific abiotic stress (various macro and micro-nutrients like P, N, and Zn) is very crucial in the coming years to improve finger millet. It will help in improving finger millet production under changing climatic conditions.

8.5.2.2 Genomic Selection and Marker-Assisted Breeding

Studies on genetic diversity and population structure are vital for characterizing the genomic selection and genetic relationships among the finger millet accessions. The genotyping, combined with genetic diversity and population structure is shown to be very potent in predicting suitable breeding materials for finger millet improvement (Dida et al. 2008). Therefore, genome-wide assessments of the genetic variation of finger millet germplasm help facilitate the use of accelerated breeding approaches through MAS. The DNA-based PCR provides the foundation for a wide range of molecular techniques to be used in genetic diversity and population

Table 8.2 Comparison of whole genome data of finger millet with model crop foxtail millet

Name of millet	Genotype	Platform used	Sequence data generated (Gb)	Genome coverage (%)	Genome size (Mb)	Total number of genes	Reference
Finger millet	ML-365	Illumina and SOLiD sequencing	45	~82	1,196	85,243	Hittalmani et al. (2017)
	PR-202	Illumina NextSeq 500, MiSeq instrument (Illumina) and PacBio RS II system	1.5	~78.2	1,189	62,348	Hatakeyama et al. (2017)
Foxtail millet	cv. Zhang gu × A10	ABI3730xl capillary sequencer; 454 FLX platform and Illumina Genome Analyzer II platform	~3.5–~4	~80	~400	24,000-29,000	Bennetzen et al. (2012)
	cv. Zhang gu × A2	Illumina second-generation sequencer	~40	~86	~423	38,801	Zhang et al. (2012)

The details such as the name of genotype, the platform used for genome sequencing, size of the sequence data, % of genome coverage, genome size, and the total number of genes are provided

Table 8.3 Details on genome and transcriptome sequences reported for finger millet under abiotic stress conditions

Type of abiotic stress	Name of the genotype	NGS platform	NCBI accession no.
Drought	PR-202	Genome assembly	PRJDB5606
	GPU-28	Transcriptome	PRJNA282859
	**	Transcriptome	PRJNA282578
	ML-365	Whole genome	PRJNA318349
	GPU-28	Transcriptome	PRJNA282860
	MR-1	Small RNA analysis	PRJNA277250
Water/moisture	**	Transcriptome	PRJNA229808
	ML-365	Transcriptome	PRJNA339512
Salinity	CO 12 and Trichy 1	Transcriptome	PRJNA236733

Details on name of abiotic stress, name of the genotype, NGS platform used and NCBI accession number are given
Source Ceasar et al. (2018)
**Details not available

structure analyses. Different molecular marker systems such as random amplified polymorphic DNA (RAPD) (Ramakrishnan et al. 2016c; Mundada et al. 2019; Kumari and Pande 2010), interspersed simple sequence repeat (ISSR) (Rajendran et al. 2016; Brhane et al. 2017), single primer amplification reaction (SPAR) (Pandian et al. 2018a), simple sequence repeat (SSR) (Dida et al. 2008; Arya et al. 2013; Babu et al. 2014b; Ramakrishnan et al. 2016b; Pandian et al. 2018b) and others have been used to characterize the genetic variation and population structure of finger millet. The polymorphism exhibited by different markers is useful for detecting the genetic variation among finger millet accessions. The variability of finger millet accessions could be utilized in breeding programs for developing abiotic stress tolerant finger millet in future.

Marker-assisted breeding has enormous potential to improve the efficiency and precision of conventional plant breeding through MAS. It is one of the important tools employed for the identification and improvement of particular traits. The DNA-based molecular markers provide a foundation for MAS, which are being widely used in the crop breeding programs. Abiotic stress tolerance is governed by QTL. Therefore, it may be possible for genetic improvement of such traits through marker-assisted breeding once QTL are identified and mapped. The microsatellite or SSR markers have been used to identify the QTL in finger millet. But, a few abiotic stress tolerant QTL have been identified in finger millet so far. Only a few QTL underlying agro-morphological and nutritional aspects were identified under different abiotic conditions (Table 8.4). To date, only association mapping has been applied to identify the QTL in finger millet for agronomically important traits. Hence, there is a crucial need to develop the linkage maps of finger millet using

Table 8.4 Details of markers associated with agronomic traits in finger millet

Mapping population	Trait	Specific traits identified	Type of marker	Name of QTL	Reference
Association mapping	Agro-morphological	Days to 50% flowering	SSR	UGEP77 and UGEP90	Babu et al. (2014a); Ramakrishnan et al. (2016a), (2017)
		Basal tiller number		UGEP98	
		Root length		UGEP9 and UGEP57	
		Seed yield		UGEP9, UGEP19 and UGEP80	
		Number of finger		UGEP75 and UGEP104	
		Number of productive tillers		SSR01, UGEP65 and UGEP98	
		Number of tillers		UGEP65 and UGEP98	
		Plant height		FM9, UGEP50	
		Flag leaf blade width		FM9	
Association mapping	Nutritional	Tryptophan	SSR	OM5 and FM8	Babu et al. (2014b); Kumar et al. (2015); Yadav et al. (2017)
		Protein		FMO2EST1	
		grain calcium content		M2, M6, M11, M16, M26, M27, M36, M45 M65 and UGEP60	

The details such as type of mapping population used, name of the traits, type of marker, and name of QTL identified are provided

superior genotypes based on phenomic selection. It could help in identification and characterization of abiotic stress tolerant QTL.

Recently, 13 QTL associated with six agronomic traits such as plant length (UGEP50), root length (UGEP9 and UGEP57), seed yield (UGEP9, UGEP19, and UGEP80), number of the finger (UGEP104 and UGEP75), number of tillers (UGEP98 and UGEP6) and productive tillers (UGEP98, UGEP65 and SSR01) were identified using 87 genomic SSR markers in 128 genotypes of finger millet (Ramakrishnan et al. 2016a, 2017). Similarly, 46 genomic SSR markers were used to analyze 190 finger millet genotypes and identify four agro-morphological traits linked to four QTL such as UGEP98 (basal tiller number), FM9 (plant height and

flag leaf blade width) and UGEP77 and UGEP90 (days to 50% flowering) (Babu et al. 2014a). Kumar et al. (2015) reported a total of nine QTL including M2, M6, M11, M16, M26, M27, M36, M45, and M65 associated with calcium content in 113 genotypes of finger millet using 23 anchored SSR markers. Furthermore, 238 genotypes were characterized using 85 genic and non-genic SSR markers and identified one QTL (UGEP69) linked to grain calcium content of finger millet (Yadav et al. 2017). Babu et al. (2014b) identified QTL associated with tryptophan content and protein content in finger millet (Table 8.4). Identification of QTL controlling agro-morphological, nutritional and abiotic stress tolerant traits along with their candidate genes could support breeding and transgenic approaches. It may be useful for finger millet crop improvement and helpful for reducing food demand in the future.

8.5.2.3 Genome-Assisted Breeding

High-throughput sequence technology like NGS facilitates rapid and accurate sequencing of many crop genomes and it helps to detect the genetic basis of phenotypic variation in crops. The complete maps of genome variations contribute to genome-wide association studies (GWAS) of multi-behavioral characteristics and functional characterization in crops. Therefore, high-resolution trait data is a crucial factor for successful GWAS. These advances will highly accelerate the study of particular crop through genome-assisted breeding. Many trait aspects related to plant growth and development have been successfully approached using GWAS in rice (Zhao et al. 2011; Famoso et al. 2011; Huang et al. 2012; Yang et al. 2014), maize (Hao et al. 2011; Xue et al. 2013), wheat (Zanke et al. 2014), sorghum (Morris et al. 2013), foxtail millet (Jia et al. 2013) and pearl millet (Saïdou et al. 2014) etc. The availability of high-resolution GWAS could be helpful for crop improvement by genome-assisted breeding programs in finger millet as well.

Notably a little attention was paid on GWAS in finger millet compared with other crops. This would crucially affect the finger millet improvement program. Genotyping by sequencing (GBS) generated single nucleotide polymorphism (SNP) markers which were used to analyze the agro-morphological traits of 113 finger millet accessions and identify reliable SNP markers linked to grain yield and its component traits (Sharma et al. 2018). In this study, five SNP sequences showed homology to candidate genes of rice and foxtail millet, which were responsible for flowering, maturity, and grain yield (Sharma et al. 2018). However, researchers need to focus more on GWAS in finger millet.

High-resolution linkage mapping with the help of GWAS identified the CCT domain-containing gene in maize (*ZmCCT*), which was homologous to the rice photoperiod response regulator *Ghd7*, a key gene affecting photoperiod response in maize (Hung et al. 2012). In similar way, GWAS identified a variety of candidate genes and their alleles for flowering time variation in rice (Zhao et al. 2011; Huang et al. 2012), maize (Hao et al. 2011; Xue et al. 2013), wheat (Le Gouis et al. 2012; Zanke et al. 2014), pearl millet (Saïdou et al. 2014) and other crops. Jia et al. (2013)

sequenced 916 diverse foxtail millet genotypes and identified 2.58 million SNPs. In the same study, Jia et al. (2013) constructed a haplotype map of the foxtail millet genome using 0.8 million common SNPs and classified the foxtail millet varieties into two divergent groups such as early and late flowering times. Furthermore, these 916 varieties of finger millet were phenotyped under five different environments and identified 512 loci associated with 47 agronomic traits through GWAS (Jia et al. 2013). GWAS are needed in finger millet for improving their agro-morphological traits which may contribute to the development of abiotic stress tolerant finger millet varieties in the future.

Similarly, GWAS for seed-related phenotypes have also been reported in major and minor cereal crops such as rice (Chen et al. 2014; Yang et al. 2014), maize (Hao et al. 2011; Xue et al. 2013), barley (Cockram et al. 2010; Pasam et al. 2012) and foxtail millet (Jia et al. 2013) etc. However, GWAS are needed for the identification of abiotic stress-tolerance related traits in finger millet in future. It may be helpful for the development of abiotic stress tolerant finger millet varieties in the future through genome-assisted breeding. Therefore, the GWAS would be a key tool of genome-assisted breeding for finger millet improvement in future.

8.5.2.4 Functional Genomics

Identification and functional characterization of valuable genes with key traits have been considered essential for developing varieties with improved traits. Therefore, functional genomics could be helpful for developing abiotic stress tolerant finger millet plants through transgenic modification. Many abiotic stress-responsive genes were identified and characterized in finger millet so far. Finger millet drought-responsive gene *EcDehydrin7* was overexpressed in transgenic tobacco (Singh et al. 2015a). The result suggests that the *EcDehydrin7* protein has a significant role in drought stress tolerance. Furthermore, seven drought-responsive genes such as *Metallothionein* (*MT*), *RISBZ4*, *Farnesylated Protein* (*ATFP6*), *Transcriptional Regulator* (*TR*), *Protein Phosphatase 2A* (*PP2A*), *Early Light Inducible Protein* (*ELIP*) and *Farnesyl Pyrophosphate Synthase* (*FPS*) were identified in leaf tissues of finger millet under different level of drought stress (Parvathi et al. 2013). Among them, *MT*, *ATFP6*, *RISBZ4*, *TR* and *PP2A* were expressed in leaves of finger millet under 60% drought stress. *FPS* and *ELIP* were expressed in leaves of finger millet under 20% and 40% drought stress, respectively. Another study showed that *TATA-box Binding Protein Associated Factor 6* (*TAF6*) was highly expressed in leaf tissues of finger millet under 20–60% drought stress (Parvathi and Nataraja 2017). Transcription factor *G-BOX BINDING FACTOR 3* (*EcGBF3*) of finger millet overexpressed in *Arabidopsis thaliana* improved tolerance to osmotic stress, drought and salinity (Ramegowda et al. 2017). Therefore, these studies provide insight into the function of drought stress-responsive genes of finger millet which could be harnessed for improvement of finger millet.

Genes responsible for nutrient transport are also identified and characterized in finger millet. Four *phosphate transporter 1* family genes (*EcPHT1;1* to *1;4*) were identified in finger millet under different regimes of inorganic phosphate (Pi) supply and colonization of arbuscular mycorrhizal fungi (AMF) (Pudake et al. 2017). Among these four genes, *EcPHT1*;*1* and *1*;*3* were highly expressed in roots and leaves of finger millet under low Pi condition. The *EcPHT1*;*4* was found in AMF colonized roots of finger millet. More recently, we have analyzed the expression pattern of 12 *PHT1* family genes (*SiPHT1*;*1* to *1*;*12*) in finger millet under low and high Pi conditions using foxtail millet gene-specific primers (Maharajan et al. 2019). In our study, *SiPHT1*;*2*, *1*;*3* and *1*;*4* were expressed in leaves of finger millet under low Pi conditions. Furthermore, *SiPHT1*;*2* and *1*;*3* were expressed in roots of finger millet under low Pi conditions (Maharajan et al. 2019). Interestingly, the expression level of *SiPHT1*;*2* and *1*;*3* were >1 fold higher in both leaf and root tissues under low Pi compared to high Pi conditions (Maharajan et al. 2019). Notably, Pudake et al. (2017) identified four *PHT1* family genes in finger millet based on the partial transcript sequences. More than 10 *PHT1* family genes have been identified in various crop plants (Nussaume et al. 2011; Baker et al. 2015; Wang et al. 2017; Roch et al. 2019). Therefore, the finger millet genome sequences (Hittalmani et al. 2017; Hatakeyama et al. 2017) will pave the way for the identification and functional characterization of all other *PHT1* family genes in finger millet.

Two contrasting finger millet genotypes [GP-45 (high calcium accumulating) and GP-1 (low calcium accumulating)] were used to understand the role of various Ca transporter family genes such as Ca^{2+}/H^{+} *antiporter* (*CAX1*), *two pore channel1* (*TPC1*), *calmodulin (CaM)-stimulated type IIB* Ca^{2+} *ATPase* and two *CaM dependent protein kinase* (*CaMK1* and *CaMK2*) (Mirza et al. 2014). Among these, *CAX1* was found in the late stages of spike development. The *TPC1* and Ca^{2+} *ATPase* were identified in the root, stem and developing spike of finger millet. This study revealed that CAX1 could be responsible for accumulating high concentrations of Ca in seeds; TPC1 and Ca^{2+} ATPase are involved in the uptake and translocation of Ca. The same group also analyzed the expression pattern of 82 Ca sensor family genes [*Calmodulin* (CaM) and *Calciuneurin B-like protein* (*CBL*); Ca^{2+} *dependent* and *CaM independent protein kinases* (*CDPKs*); *SOS3/CBL interacting protein kinases* (*SIPKs/CIPKs*); *CaM dependent protein kinases* (*CaMKs*); Ca^{2+}*/CaM dependent protein kinases* (*CCaMKs*); *CDPK related protein kinases* (*CRKs*); *phosphoenolpyruvate* (*PEP*) and *carboxylase kinase-related kinases* (*PEPRKs*)] in the developing spikes of GP-1 and GP-45 genotypes of finger millet (Singh et al. 2014). The results of transcriptome analysis revealed that 24 genes (seven encoded for *CaML*, 2-*CRK*, 5-*CBL*, 7-*CIPK* and 4-*CDPK*) and 11 genes (five encoded for *CaML*, 2-*CRK*, 3-*CIPK*, and 1-*CDPK*) were highly expressed in the developing spikes of GP-45 and GP-1 genotypes respectively. Interestingly, *EcCIPK9* was highly expressed in the developing spike of GP-45 when compared to the GP-1 genotype (Singh et al. 2014). The same group also

identified 19 Ca^{2+} transporter family genes (11 for *Ca^{2+} ATPases*, seven Ca^{2+}/cation exchangers and one Ca^{2+} channel) in developing spikes of these two finger millet genotypes (Singh et al. 2015b). Moreover, Chinchole et al. (2017) found that *EcCIPK24* gene was highly expressed in root, stem and leaf tissues of the GP45 genotype compared to the GP1 genotype. This study suggests that *EcCIPK24* can play an important role in high seed Ca accumulation (Chinchole et al. 2017). Therefore, the identification and characterization of other nutrient transporter will be helpful for development of transgenic finger millet which may be helpful to reduce the effects of nutrient-related stress.

Rahman et al. (2014) identified many salinity stress-responsive genes in leaves of two contrasting finger millet genotypes [Co-12 (susceptible) and Trichy 1 (tolerant)] under salinity condition through RNAseq. Later, the same group also stated that overexpression of *EcNAC67* gene in rice enhanced salinity and drought tolerances. Similarly, overexpression of *EcNAC1* in tobacco exhibited tolerance to various abiotic stresses like drought, osmotic stress and salinity (Ramegowda et al. 2012). These two studies indicated that the *EcNAC* can be used as a novel gene for engineering tolerance against drought and salinity stress in crop plants (Rahman et al. 2016). Furthermore, basic leucine zippers family (bZIP) (*EcbZIP60)* and *basic helix-loop-helix* (bHLH) family genes (*EcbHLH57*) were analyzed in leaves of finger millet under drought, osmotic, salt and methyl viologen-induced stress (Babitha et al. 2015a, b). These findings could help in the improvement of finger millet under salinity stress conditions.

The transcription factor of *prolamin-binding factor DNA binding with one finger only* (*PBF Dof*) could be an important regulator for seed storage protein gene expression. Expression pattern of *PBF Dof* was analyzed in various tissues like root, stem and flag leaf at the vegetative stage and developing spikes of three-finger millet genotypes [PRM-1 (brown), PRM-701 (golden), and PRM-801 (white)] with differing seed protein content and color (Gupta et al. 2011). Interestingly, the expression of *PBF Dof* was higher in developing spikes compared to root, stem and flag leaf in all three genotypes. Likewise, six genes (*E. coracana high-affinity nitrate transporter (EcHNRT2)*, *E. coracana low-affinity nitrate transporter (EcLNRT1)*, *E. coracana nitrate reductase (EcNADH-NR)*, *E. coracana glutamine synthetase (EcGS)*, *E. coracana glutamine oxoglutarate aminotransferase (EcFd-GOGAT*) and *EcDof1* involved in nitrate uptake and assimilation were studied in two contrasting finger millet genotypes [GE-1437 (low-protein content) and GE-3885 (high-protein content)] (Gupta et al. 2013). Among them, *EcLNRT1, EcNADH-NR, EcGS, EcFd-GOGAT* and*EcDof1* were expressed in the leaves of GE-3885 under N deficiency. Furthermore, compared to GE-1437, expression of *EcHNRT2* was also strongly induced in both roots and shoots of GE-3885 genotype under low N conditions (Gupta et al. 2013). This study indicates that high protein content genotype is a quick sensor of N compared with the low protein content genotype (Gupta et al. 2013). The same group also analyzed the expression pattern of *EcDof1* and *EcDof2* in the root and shoot tissues of the same two genotypes (Gupta et al. 2014) under low and high N conditions. The *EcDof2* expression level was higher in shoots of GE-1437 under low N compared to GE-3885. *EcDof1*

expression level was higher in roots of GE-3885 under high N conditions compared to the GE-1437. However, the *EcDof1*/*EcDof2* ratio was higher in the roots of GE-3885 than in GE-3885 (Gupta et al. 2014).

Functional genomics studies could help in the management of abiotic stress in finger millet. However, more in-depth research is needed for the identification and validation of candidate genes for abiotic stress tolerance for further breeding and transgenic approaches. The released finger millet genome sequences will pave the way for the identification and functional characterization of more abiotic stress-responsive genes in finger millet. This will help in the development of improved varieties in finger millet and may provide food and nutritional security in future.

8.6 Conclusion and Future Prospects

Rapidly growing populations put more pressure on the agricultural sector to produce more food from available land. Global climate changes throw new challenges to develop climate resilient crops. The developing nations of the world will have to face demand for more food and take efforts to tackle malnutrition in the coming decades. Therefore, finger millet is considered as a nutria-cereal or a nutraceutical crop that is seen as a solution to malnutrition and hidden hunger around the world. However, abiotic constraints affect finger millet production badly. They reduce crop productivity and nutritional quality. So, there is an urgent need to improve finger millet to overcome abiotic constraints. With the advance in genomics and molecular marker technology, a new era of molecular breeding has emerged that has gradually accelerated the pace of plant breeding. Also, plant breeding has gradually changed from phenotype-based to genotype-based selection. Therefore, genome-assisted breeding approaches are helpful for the crop improvement. It is a long-term solution to improve crops with desirable traits compared to other approaches. In finger millet many QTL related to agronomic and nutritional aspects were identified so far. It could be helpful for the development of improved varieties of finger millet through genome-assisted breeding. The genome-based population study is still lacking in finger millet. So far, only association mapping populations were used to identify agronomically important QTL. There is an urgent need to develop linkage mapping of traits related to multi-abiotic stress constraints in the coming years. This may help in the identification of novel abiotic stress tolerant QTL for molecular breeding for millet improvements. Notably, very few works are available on GWAS in finger millet compared with other crops. Therefore, researchers need to focus on this area. In future, the GWAS could provide a key means of genome-assisted breeding for finger millet improvement. Also, identification and functional characterization of abiotic stress-responsive candidate genes will help in the development of improved varieties of finger millet. Available finger millet genomic and transcriptome sequences will serve as a base for functional genomics studies in future. It may help for finger millet improvement and provide food and nutritional security in the future.

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References

Alloway B (2009) Soil factors associated with zinc deficiency in crops and humans. Environ Geochem Health 31(5):537–548

Anjaneyulu E, Reddy P, Sunita M, Kishor P, Meriga B (2014) Salt tolerance and activity of antioxidative enzymes of transgenic finger millet overexpressing a vacuolar HC-pyrophosphatase gene (SbVPPase) from *Sorghum bicolor*. J Plant Physiol 171:789–798

Arya L, Verma M, Gupta V, Seetharam A (2013) Use of genomic and genic SSR markers for assessing genetic diversity and population structure in Indian and African finger millet (*Eleusine coracana* (L.) Gaertn.) germplasm. Plant Syst Evol 299(7):1395–1401

Babitha K, Ramu S, Nataraja KN, Sheshshayee M, Udayakumar M (2015a) 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(9):181

Babitha K, Vemanna RS, Nataraja KN, Udayakumar M (2015b) Overexpression of EcbHLH57 transcription factor from *Eleusine coracana* L. in tobacco confers tolerance to salt, oxidative and drought stress. PLoS One 10(9):e0137098

Babu BK, Singh U, Yadav S, Kumar A (2013) Molecular marker technology for finger millet crop improvement—an under-utilized, food and nutritional security crop. Biotechnol Today 3 (2):57–60

Babu BK, Agrawal P, Pandey D, Jaiswal J, Kumar A (2014a) Association mapping of agro-morphological characters among the global collection of finger millet genotypes using genomic SSR markers. Mol Biol Rep 41(8):5287–5297

Babu BK, Agrawal P, Pandey D, Kumar A (2014b) Comparative genomics and association mapping approaches for opaque2 modifier genes in finger millet accessions using genic, genomic and candidate gene-based simple sequence repeat markers. Mol Breed 34(3):1261–1279

Baker A, Ceasar SA, Palmer AJ, Paterson JB, Qi W, Muench SP, Baldwin SA (2015) Replace, reuse, recycle: improving the sustainable use of phosphorus by plants. J Exp Bot 66(12):3523–3540

Bansal KC, Lenka SK, Mondal TK (2014) Genomic resources for breeding crops with enhanced abiotic stress tolerance. Plant Breed 133(1):1–11

Beddington J (2010) Food security: contributions from science to a new and greener revolution. Phil TransRoy Soc B: Biol Sci 365(1537):61–71

Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, Estep M, Feng L, Vaughn JN, Grimwood J (2012) Reference genome sequence of the model plant Setaria. Nat Biotechnol 30(6):555

Bhatt D, Negi M, Sharma P, Saxena SC, Dobriyal AK, Arora S (2011) Responses to drought induced oxidative stress in five finger millet varieties differing in their geographical distribution. Physiol Mol Biol Plants 17(4):347

Brhane H, Haileselassie T, Tesfaye K (2017) Genetic diversity and population structure of Ethiopian finger millet (*Eleusine coracana* (L.) Gaertn) genotypes using inter simple sequence repeat (ISSR) markers. Afr J Biotechnol 16(21):1203–1209

Ceasar AS, Maharajan T, Ajeesh Krishna T, Ramakrishnan M, Victor Roch G, Satish L, Ignacimuthu S (2018) Finger millet [*Eleusine coracana* (L.) Gaertn.] improvement: current status and future interventions of whole genome sequence. Front Plant Sci 9:1054

Chen H, Xie W, He H, Yu H, Chen W, Li J, Yu R, Yao Y, Zhang W, He Y (2014) A high-density SNP genotyping array for rice biology and molecular breeding. Mol Plant 7(3):541–553

Chinchole M, Pathak RK, Singh UM, Kumar A (2017) Molecular characterization of EcCIPK24 gene of finger millet (*Eleusine coracana*) for investigating its regulatory role in calcium transport. 3 Biotechnol 7(4):267

Cockram J, White J, Zuluaga DL, Smith D, Comadran J, Macaulay M, Luo Z, Kearsey MJ, Werner P, Harrap D (2010) Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. Proc Natl Acad Sci USA 107(50):21611–21616

Dasanayaka P (2016) Characterization of some ex situ conserved finger millet (*Eleusine coracana* (L.)) germplasm accessions in Sri Lanka. Intl J Interdiscip Multidiscip Stud 3(2):141–150

Dhanalakshmi T, Ramesh S, Upadhyaya H, Rao A, Gangappa E, Priyadarshini S (2014) Genetic variability for morpho-agronomic traits in core germplasm collections of finger millet (*Eleusine coracana* (L.) Gaertn.) based on third and fourth degree statistics and their origin. Intl J Trop Agric 32(1/2):239–242

Dida MM, Wanyera N, Dunn MLH, Bennetzen JL, Devos KM (2008) Population structure and diversity in finger millet (*Eleusine coracana*) germplasm. Trop Plant Biol 1(2):131–141

Famoso AN, Zhao K, Clark RT, Tung C-W, Wright MH, Bustamante C, Kochian LV, McCouch SR (2011) Genetic architecture of aluminum tolerance in rice (*Oryza sativa*) determined through genome-wide association analysis and QTL mapping. PLoS Genet 7(8):

Goron TL, Raizada MN (2015) Genetic diversity and genomic resources available for the small millet crops to accelerate a new green revolution. Front Plant Sci 6:157

Goron TL, Bhosekar VK, Shearer CR, Watts S, Raizada MN (2015) Whole plant acclimation responses by finger millet to low nitrogen stress. FrontPlant Sci 6:652

Goswami A, Prasad B, Joshi V (2015) Characterization of finger millet [*Eleusine coracana* (L.) Gaertn.] germplasm for morphological parameters under field conditions. J Appl Natl Sci 7 (2):836–838

Gupta N, Gupta AK, Singh N, Kumar A (2011) Differential expression of PBF Dof transcription factor in different tissues of three finger millet genotypes differing in seed protein content and color. Plant Mol Biol Rep 29(1):69–76

Gupta AK, Gaur VS, Gupta S, Kumar A (2013) Nitrate signals determine the sensing of nitrogen through differential expression of genes involved in nitrogen uptake and assimilation in finger millet. Funct Integr Genom 13(2):179–190

Gupta S, Gupta SM, Gupta AK, Gaur VS, Kumar A (2014) Fluctuation of Dof1/Dof2 expression ratio under the influence of varying nitrogen and light conditions: involvement in differential regulation of nitrogen metabolism in two genotypes of finger millet (*Eleusine coracana* L.). Gene 546(2):327–335

Gupta SM, Arora S, Mirza N, Pande A, Lata C, Puranik S, Kumar J, Kumar A (2017) Finger millet: a "certain" crop for an "uncertain" future and a solution to food insecurity and hidden hunger under stressful environments. Front Plant Sci 8:643

Hao Z, Li X, Xie C, Weng J, Li M, Zhang D, Liang X, Liu L, Liu S, Zhang S (2011) Identification of functional genetic variations underlying drought tolerance in maize using SNP markers. J Integr Plant Biol 53(8):641–652

Haradari C, Gowda J, Ugalat J (2012) Formation of core set in Indian and African finger millet [*Eleusine coracana* (L.) Gaertn] germplasm accessions. Indian J Genet 72(3):358

Hatakeyama M, Aluri S, Balachadran MT, Sivarajan SR, Patrignani A, Grüter S, Poveda L, Shimizu-Inatsugi R, Baeten J, Francoijs K-J (2017) Multiple hybrid de novo genome assembly of finger millet, an orphan allotetraploid crop. DNA Res 25(1):39–47

Hema R, Vemanna RS, Sreeramulu S, Reddy CP, Senthil-Kumar M, Udayakumar M (2014) Stable expression of mtlD gene imparts multiple stress tolerance in finger millet. PLoS ONE 9(6):

Hiremath S, Salimath S (1992) The 'A' genome donor of *Eleusine coracana* (L.) Gaertn. (Gramineae). Theor Appl Genet 84(5–6):747–754

Hittalmani S, Mahesh H, Shirke MD, Biradar H, Uday G, Aruna Y, Lohithaswa H, Mohanrao A (2017) Genome and transcriptome sequence of finger millet (*Eleusine coracana* (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics 18 (1):465

Huang X, Zhao Y, Li C, Wang A, Zhao Q, Li W, Guo Y, Deng L, Zhu C, Fan D (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nat Genet 44(1):32–39

Hung H-Y, Shannon LM, Tian F, Bradbury PJ, Chen C, Flint-Garcia SA, McMullen MD, Ware D, Buckler ES, Doebley JF (2012) ZmCCT and the genetic basis of day-length adaptation underlying the post domestication spread of maize. Proc Natl Acad Sci USA 109(28):E1913–E1921

Isingoma BE, Mbugua S, Karuri E, Maina G (2015) Improving the nutritional value of traditional finger millet porridges for children aged 7–24 months in Bujenje County of Western Uganda. Afr J Food Sci 9(8):426–436

Jia G, Huang X, Zhi H, Zhao Y, Zhao Q, Li W, Chai Y, Yang L, Liu K, Lu H (2013) A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (*Setaria italica*). Nat Genet 45(8):957–961

Joshi H, Katoch K (1990) Nutritive value of millets: a comparison with cereals and pseudocereals. Himal Res Dev 9:26–28

Krishna TPA, Ceasar S, Maharajan T, Ramakrishnan M, Duraipandiyan V, Al-Dhabi N, Ignacimuthu S (2017) Improving the zinc-use efficiency in plants: a review. SABRAO J Breed Genet 49(3):1–20

Krishna TPA, Maharajan T, David RHA, Ramakrishnan M, Ceasar SA, Duraipandiyan V, Roch GV, Ignacimuthu S (2018) Microsatellite markers of finger millet (*Eleusine coracana* (L.) Gaertn) and foxtail millet (*Setaria italica* (L.) Beauv) provide resources for cross-genome transferability and genetic diversity analyses in other millets. Biocatal Agric Biotechnol 16:493–501

Krishna TPA, Maharajan T, Roch GV, Duraipandiyan V, Ignacimuthu S (2020a) Microsatellite marker: importance and implications of cross-genome analysis for finger millet (*Eleusine coracana* (L.) Gaertn). Curr Biotechnol 9(1):1–12

Krishna TPA, Maharajan T, Roch GV, Ramakrishnan M, Ceasar SA, Ignacimuthu S (2020b) Hybridization and hybrid detection through molecular markers in finger millet [*Eleusine coracana* (L.) Gaertn.]. J Crop Improv 34(3):335–355

Krishnamurthy L, Upadhyaya HD, Purushothaman R, Gowda CLL, Kashiwagi J, Dwivedi SL, Singh S, Vadez V (2014) The extent of variation in salinity tolerance of the minicore collection of finger millet (*Eleusine coracana* L. Gaertn.) germplasm. Plant Sci 227:51–59

Krishnamurthy L, Upadhyaya HD, Kashiwagi J, Purushothaman R, Dwivedi SL, Vadez V (2016) Variation in drought-tolerance components and their interrelationships in the minicore collection of finger millet germplasm. Crop Sci 56(4):1914–1926

Kumar M (2013) Crop plants and abiotic stresses. J Biomol Res Ther 3(1):e125

Kumar A, Yadav S, Panwar P, Gaur V, Sood S (2015) Identification of anchored simple sequence repeat markers associated with calcium content in finger millet (*Eleusine coracana*). Proc Natl AcadS ci India Sect B: Biol Sci 85(1):311–317

Kumar A, Metwal M, Kaur S, Gupta AK, Puranik S, Singh S, Singh M, Gupta S, Babu BK, Sood S (2016) Nutraceutical value of finger millet [*Eleusine coracana* (L.) Gaertn.], and their improvement using omics approaches. Front Plant Sci 7:934

Kumari K, Pande A (2010) Study of genetic diversity in finger millet (*Eleusine coracana* L. Gaertn) using RAPD markers. Afr J Biotechnol 9(29):4542–4549

Le Gouis J, Bordes J, Ravel C, Heumez E, Faure S, Praud S, Galic N, Remoue C, Balfourier F, Allard V (2012) Genome-wide association analysis to identify chromosomal regions determining components of earliness in wheat. Theor Appl Genet 124(3):597–611

Leng P-f, Lübberstedt T, Xu M-l (2017) Genomics-assisted breeding—a revolutionary strategy for crop improvement. J Integr Agric 16(12):2674–2685

Maharajan T, Ceasar SA, Krishna TPA, Ramakrishnan M, Duraipandiyan V, Naif Abdulla AD, Ignacimuthu S (2018) Utilization of molecular markers for improving the phosphorus efficiency in crop plants. Plant Breed 137(1):10–26

Maharajan T, Ceasar SA, Krishna TPA, Ignacimuthu S (2019) Phosphate supply influenced the growth, yield and expression of PHT1 family phosphate transporters in seven millets. Planta 250(5):1433–1448

Mathanghi S, Sudha K (2012) Functional and phytochemical properties of finger millet (*Eleusine coracana*) for health. Intl J Pharmaceut Chem Biol Sci 2(4):431–438

Mathur P (2012) Global strategy for the *ex situ* conservation of finger millet and its wild relatives. Global Crop Diversity Trust & ICRISAT, pp 7–57

Mibithi-Mwikya S, Ooghe W, Van Camp J, Nagundi D, Huyghebaert A (2000) Amino acid profile after sprouting, autoclaving and lactic acid fermentation of finger millet (*Elusine coracana*) and kidney beans (*Phaseolus vulgaris* L.). J Agric Food Chem 48:3081–3085

Mirza N, Taj G, Arora S, Kumar A (2014) Transcriptional expression analysis of genes involved in regulation of calcium translocation and storage in finger millet (*Eleusine coracana* L. Gartn.). Gene 550(2):171–179

Morris GP, Rhodes DH, Brenton Z, Ramu P, Thayil VM, Deshpande S, Hash CT, Acharya C, Mitchell SE, Buckler ES (2013) Dissecting genome-wide association signals for loss-of-function phenotypes in sorghum flavonoid pigmentation traits. G3: Genes Genom Genet 3(11):2085–2094

Mukami A, Ngetich A, Mweu C, Oduor RO, Muthangya M, Mbinda WM (2019) Differential characterization of physiological and biochemical responses during drought stress in finger millet varieties. Physiol Mol Biol Plants 25(4):837–846

Mukami A, Ng'etich A, Syombua E, Oduor R, Mbinda W (2020) Varietal differences in physiological and biochemical responses to salinity stress in six finger millet plants. Physiol Mol Biol Plants 1–14

Mundada PS, Umdale SD, Nikam TD, Ahire ML (2019) Genetic diversity using RAPD markers, mineral composition and their correlation in selected local landraces of finger millet [*Eleusine coracana* (L.) Gaertn.]. Vegetos 32(1):1–10

National Research Council (1996) Lost crops of Africa. Vol 1. Grains. National Academy Press, Washington DC, p 383

Nirgude M, Babu BK, Shambhavi Y, Singh U, Upadhyaya H, Kumar A (2014) Development and molecular characterization of genic molecular markers for grain protein and calcium content in finger millet (*Eleusine coracana* (L.) Gaertn.). Mol Biol Rep 41(3):1189–1200

Nussaume L, Kanno S, Javot H, Marin E, Nakanishi TM, Thibaud M-C (2011) Phosphate import in plants: focus on the PHT1 transporters. Front Plant Sci 2:83

Onyango AO (2016) Finger millet: food security crop in the arid and semi-arid lands (ASALs) of Kenya. World Environ 6(2):62–70

Pandian S, Marichelvam K, Satish L, Ceasar SA, Pandian SK, Ramesh M (2018a) SPAR markers-assisted assessment of genetic diversity and population structure in finger millet (*Eleusine Coracana* (L.) Gaertn) mini-core collection. J Crop Sci Biotechnol 21(5):469–481

Pandian S, Satish L, Rameshkumar R, Muthuramalingam P, Rency AS, Rathinapriya P, Ramesh M (2018b) Analysis of population structure and genetic diversity in an exotic germplasm collection of *Eleusine coracana* (L.) Gaertn. using genic-SSR markers. Gene 653:80–90

Parvathi M, Nataraja KN (2017) Discovery of stress responsive TATA-box binding protein associated Factor6 (TAF6) from finger millet (*Eleusine coracana* (L.) Gaertn). J Plant Biol 60 (4):335–342

Parvathi M, Nataraja KN, Yashoda B, Ramegowda H, Mamrutha H, Rama N (2013) Expression analysis of stress responsive pathway genes linked to drought hardiness in an adapted crop, finger millet (*Eleusine coracana*). J Plant Biochem Biotechnol 22(2):193–201

Pasam RK, Sharma R, Malosetti M, van Eeuwijk FA, Haseneyer G, Kilian B, Graner A (2012) Genome-wide association studies for agronomical traits in a world wide spring barley collection. BMC Plant Biol 12(1):16

Poonia K, Chavan S, Daniel M (2012) Fixed oil composition, polyphenols and phospholipids of finger millet [*Eleusine Coracana* (L.) Gaertn]. Biol Forum Intl J 4(1):45–47

Pore M, Magar N (1979) Nutrient composition of hybrid varieties of finger millet [*Eleusine coracana* (Linn.) Gaertn., India]. Indian J Agric Sci 49(7):526–531

Pudake RN, Mehta CM, Mohanta TK, Sharma S, Varma A, Sharma AK (2017) Expression of four phosphate transporter genes from Finger millet (*Eleusine coracana* L.) in response to mycorrhizal colonization and Pi stress. 3 Biotechnol 7(1):17

Puranik S, Kam J, Sahu PP, Yadav R, Srivastava RK, Ojulong H, Yadav R (2017) Harnessing finger millet to combat calcium deficiency in humans: challenges and prospects. Front Plant Sci 8:1311

Rahman H, Jagadeeshselvam N, Valarmathi R, Sachin B, Sasikala R, Senthil N, Sudhakar D, Robin S, Muthurajan R (2014) Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (*Eleusine coracana* L.) through RNA-sequencing. Plant Mol Biol 85 (4–5):485–503

Rahman H, Ramanathan V, Nallathambi J, Duraialagaraja S, Muthurajan R (2016) Over-expression of a NAC 67 transcription factor from finger millet (*Eleusine coracana* L.) confers tolerance against salinity and drought stress in rice. BMC Biotechnol 16(1):35

Rajendran HAD, Muthusamy R, Stanislaus AC, Krishnaraj T, Kuppusamy S, Ignacimuthu S, Al-Dhabi NA (2016) Analysis of molecular variance and population structure in southern Indian finger millet genotypes using three different molecular markers. J Crop Sci Biotechnol 19(4):275–283

Ramakrishnan M, Antony Ceasar S, Duraipandiyan V, Vinod K, Kalpana K, Al-Dhabi N, Ignacimuthu S (2016a) Tracing QTLs for leaf blast resistance and agronomic performance of finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes through association mapping and in silico comparative genomics analyses. PLoS One 11(7):e0159264

Ramakrishnan M, Ceasar SA, Duraipandiyan V, Al-Dhabi N, Ignacimuthu S (2016b) Assessment of genetic diversity, population structure and relationships in Indian and non-Indian genotypes of finger millet (*Eleusine coracana* (L.) Gaertn) using genomic SSR markers. Springer Plus 5 (1):120

Ramakrishnan M, Ceasar SA, Duraipandiyan V, Al-Dhabi N, Ignacimuthu S (2016c) Using molecular markers to assess the genetic diversity and population structure of finger millet (*Eleusine coracana* (L.) Gaertn.) from various geographical regions. Genet Res Crop Evol 63 (2):361–376

Ramakrishnan M, Ceasar SA, Vinod K, Duraipandiyan V, Ajeesh Krishna T, Upadhyaya HD, Al-Dhabi N, Ignacimuthu S (2017) Identification of putative QTLs for seedling stage phosphorus starvation response in finger millet (*Eleusine coracana* L. Gaertn.) by association mapping and cross species synteny analysis. PLoS One 12(8):e0183261

Ramegowda V, Senthil-Kumar M, Nataraja KN, Reddy MK, Mysore KS, Udayakumar M (2012) Expression of a finger millet transcription factor, EcNAC1, in tobacco confers abiotic stress-tolerance. S 7(7):

Ramegowda V, Gill US, Sivalingam PN, Gupta A, Gupta C, Govind G, Nataraja KN, Pereira A, Udayakumar M, Mysore KS (2017) GBF3 transcription factor imparts drought tolerance in *Arabidopsis thaliana*. Sci Rep 7(1):1–13

Rashid A, Ryan J (2004) Micronutrient constraints to crop production in soils with Mediterranean-type characteristics: a review. J Plant Nutri 27(6):959–975

Ray DK, West PC, Clark M, Gerber JS, Prishchepov AV, Chatterjee S (2019) Climate change has likely already affected global food production. PLoS ONE 14(5):

Reddy VG, Upadhyaya H, Gowda C, Singh S (2009) Characterization of eastern African finger millet germplasm for qualitative and quantitative characters at ICRISAT. JSAT Agric Res 7:1–9

Rejeb IB, Pastor V, Mauch-Mani B (2014) Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. Plants 3(4):458–475

Roch VG, Maharajan T, Ceasar SA, Ignacimuthu S (2019) The role of PHT1 family transporters in the acquisition and redistribution of phosphorus in plants. Crit Rev Plant Sci 38(3):171–198

Safaa A, Fattah AEM (2007) Effect of nitrogen forms on nitrate contents and mineral composition in lettuce plants in sandy and calcareous soils. J Appl Sci Res 3(11):1630–1636

Saha D, Gowda MC, Arya L, Verma M, Bansal KC (2016) Genetic and genomic resources of small millets. Crit Rev Plant Sci 35(1):56–79

Saïdou A-A, Clotault J, Couderc M, Mariac C, Devos KM, Thuillet A-C, Amoukou IA, Vigouroux Y (2014) Association mapping, patterns of linkage disequilibrium and selection in the vicinity of the PHYTOCHROME C gene in pearl millet. Theor Appl Genet 127(1):19–32

Sharma D, Tiwari A, Sood S, Jamra G, Singh N, Meher PK, Kumar A (2018) Genome wide association mapping of agro-morphological traits among a diverse collection of finger millet (*Eleusine coracana* L.) genotypes using SNP markers. PLoS One 13(8):e0199444

Shibairo S, Madegwa Y, Onwonga R (2016) Finger millet (*Eleusine corocona*) production in Lower Eastern, Kenya: status, constraints and opportunities. Brit J Appl Sci Technol 14(6):1

Shobana S, Krishnaswamy K, Sudha V, Malleshi N, Anjana R, Palaniappan L, Mohan V (2013) Finger millet (Ragi, *Eleusine coracana* L.): a review of its nutritional properties, processing, and plausible health benefits. In: Jeyakumar H (ed) Advances in food and nutrition research, vol 69. Elsevier, USA, pp 1–39

Singh P, Raghuvanshi RS (2012) Finger millet for food and nutritional security. Afr J Food Sci 6 (4):77–84

Singh UM, Chandra M, Shankhdhar SC, Kumar A (2014) Transcriptome wide identification and validation of calcium sensor gene family in the developing spikes of finger millet genotypes for elucidating its role in grain calcium accumulation. PLoS ONE 9(8):

Singh RK, Singh VK, Raghavendrarao S, Phanindra MLV, Raman KV, Solanke AU, Kumar PA, Sharma TR (2015a) Expression of finger millet EcDehydrin7 in transgenic tobacco confers tolerance to drought stress. Appl Biochem Biotechnol 177(1):207–216

Singh UM, Metwal M, Singh M, Taj G, Kumar A (2015b) Identification and characterization of calcium transporter gene family in finger millet in relation to grain calcium content. Gene 566 (1):37–46

Sood S, Kumar A, Babu BK, Gaur VS, Pandey D, Kant L, Pattnayak A (2016) Gene discovery and advances in finger millet [*Eleusine coracana* (L.) Gaertn.] genomics—an important nutri-cereal of future. Front Plant Sci 7:1634

Sood S, Joshi DC, Chandra AK, Kumar A (2019) Phenomics and genomics of finger millet: current status and future prospects. Planta 250(3):1–21

Sripriya G, Antony U, Chandra T (1997) Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). Food Che 58(4):345–350

Subash N, Mohan HR, Banukumar K (2011) Comparing water-vegetative indices for rice (*Oryza sativa* L.)–wheat (*Triticum aestivum* L.) drought assessment. Comput Electron Agric 77 (2):175–187

Suman A, Surin S, Ahmad E (2019) Finger millet germplasm characterization and evaluation using principal component analysis. Intl J Chem Stud 7:1002–1005

Swaminaidu N, Ghosh S, Mallikarjuna K (2015) Millets: the miracle grain. Intl J Pharma Bio Sci 6 (4):440–446

Talwar HS, Kumar S, Madhusudhana R, Nanaiah GK, Ronanki S, Tonapi VA (2020) Variations in drought tolerance components and their association with yield components in finger millet (*Eleusine coracana*). Funct Plant Biol 47(7):659–674

Tesfaye K, Mengistu S (2017) Phenotypic characterization of Ethiopian finger millet accessions (*Eleusine coracana* (L.) Gaertn), for their agronomically important traits. Acta Universit Sapien Agric Environ 9(1):107–118

Thapliyal V, Singh K (2015) Finger millet: potential millet for food security and power house of nutrients. Intl Res Agric Forest 2(2):22–33

Ulaganathan V, Nirmalakumari A (2015) Finger millet germplasm characterization and evaluation using principal component analysis. SABRAO J Breed Genet 47(2):79–88

Umar I, Kwon-Ndung E (2014) Assessment of variability of finger millet (*Eleusine coracana* (L) Gaertn) landraces germplasm in Northern Nigeria. Niger J Genet 28(2):48–51

Upadhyaya H, Gowda C, Reddy VG (2007) Morphological diversity in finger millet germplasm introduced from Southern and Eastern Africa. J SAT Agric Res 3(1):1–3

Vetriventhan M, Azevedo VC, Upadhyaya H, Nirmalakumari A, Kane-Potaka J, Anitha S, Ceasar SA, Muthamilarasan M, Bhat BV, Hariprasanna K (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63:217–239

Wang D, Lv S, Jiang P, Li Y (2017) Roles, regulation, and agricultural application of plant phosphate transporters. Front Plant Sci 8:817

Xue Y, Warburton ML, Sawkins M, Zhang X, Setter T, Xu Y, Grudloyma P, Gethi J, Ribaut J-M, Li W (2013) Genome-wide association analysis for nine agronomic traits in maize under well-watered and water-stressed conditions. Theor Appl Genet 126(10):2587–2596

Yadav S, Kumar, Thakur A (2017) Marker-trait association analysis for grain calcium content in global collection of finger millet genotypes. J Food Process Technol (8)1:75

Yamunarani R, Govind G, Ramegowda V, Thammegowda HV, Guligowda SA (2016) Genetic diversity for grain Zn concentration in finger millet genotypes: potential for improving human Zn nutrition. Crop J 4(3):229–234

Yang W, Guo Z, Huang C, Duan L, Chen G, Jiang N, Fang W, Feng H, Xie W, Lian X (2014) Combining high-throughput phenotyping and genome-wide association studies to reveal natural genetic variation in rice. NatCommun 5:5087

Zanke C, Ling J, Plieske J, Kollers S, Ebmeyer E, Korzun V, Argillier O, Stiewe G, Hinze M, Beier S (2014) Genetic architecture of main effect QTL for heading date in European winter wheat. Front Plant Sci 5:217

Zhang G, Liu X, Quan Z, Cheng S, Xu X, Pan S, Xie M, Zeng P, Yue Z, Wang W (2012) Genome sequence of foxtail millet (*Setaria italica*) provides insights into grass evolution and biofuel potential. NatBiotechnol 30(6):549–554

Zhao K, Tung C-W, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. NatCommun 2(1):1–10

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci USA 114(35):9326–9331