Chittaranjan Kole *Editor*

Genomic Designing for Abiotic Stress Resistant Oilseed Crops

Springer

Genomic Designing for Abiotic Stress Resistant Oilseed Crops

Chittaranjan Kole
Editor

Genomic Designing for Abiotic Stress Resistant Oilseed Crops

Springer

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

ISBN 978-3-030-90043-4 ISBN 978-3-030-90044-1 (eBook)
https://doi.org/10.1007/978-3-030-90044-1

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Switzerland AG 2022
This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Dedicated to

Dr. (Mrs.) Manju Sharma

An eminent Indian scientist and administrator of several scientific research and policy-making bodies in India; former Secretary, to the Government of India Department of Biotechnology; and Distinguished Women Scientist Chair, The National Academy of Sciences, Allahabad, India

With regards & gratitude for her generous appreciations of my scientific contributions and her strong support and encouragement during my training, research and administration in the field of plant genomics and biotechnology!

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^5needs—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 F^5 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 Oilseed Crops*" includes eight chapters focused on Soybean, Rapeseed, Sunflower, Peanut, Rape and Mustard, Sesame, Castor and Flax contributed by 58 scientists from six countries Canada, China, India, Japan, Serbia, and USA. 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

Contents

1 Genomic Designing for Abiotic Stress Tolerant Soybean 1
Milind B. Ratnaparkhe, Gyanesh K. Satpute, Giriraj Kumawat, Subhash Chandra, Viraj G. Kamble, Rucha Kavishwar, Vijayata Singh, Jogendra Singh, Ajay K. Singh, S. V. Ramesh, Virender Kumar, Sreeja Sudhakaran, Manoj K. Srivastava, Nishtha Shesh, Anjana Jajoo, Sanjay Gupta, Maharaj Singh, Donghe Xu, Madan Bhattacharya, and Henry T. Nguyen

2 Designing the Rapeseed Genome for Abiotic Stress Tolerance 75
Inderpreet Dhaliwal, Indu Rialch, Kusum Rana, Jasmeet Kaur, and Gurpreet Kaur

3 Sunflower and Abiotic Stress: Genetics and Breeding for Resistance in the—Omics Era Sunflower Abiotic Stress Breeding 101
Nada Hladni, Chao-Chien Jan, Milan Jocković, Sandra Cvejić, Siniša Jocić, Aleksandra Radanović, and Dragana Miladinović

4 Integration of Genomics Approaches in Abiotic Stress Tolerance in Groundnut (*Arachis hypogaea* L.): An Overview 149
B. Aravind, Spurthi N. Nayak, Rakeshkumar S. Choudhary, Spoorti S. Gandhadmath, P. V. V. Prasad, Manish K. Pandey, Ramesh S. Bhat, Naveen Puppala, Putta Latha, Palagiri Sudhakar, and Rajeev K. Varshney

5 Drought Tolerance in Rapeseed-Mustard: Conventional and Molecular Approaches 199
Maharaj Singh, V. V. Singh, Naveen Singh, and Monika

6 Genomic Designing for Sesame Resistance to Abiotic Stresses 219
Xiurong Zhang, Jun You, Hongmei Miao, and Haiyang Zhang

7 Abiotic Stresses in Castor Plant 235
Xuegui Yin, Jiannong Lu, Akwasi Yeboah, and Yuelian Liu

8 Designing Genomic Solutions to Enhance Abiotic Stress Resistance in Flax 251
Nadeem Khan, Frank M. You, and Sylvie Cloutier

Contributors

B. Aravind Department of Biotechnology, University of Agricultural Sciences, Dharwad, India

Ramesh S. Bhat Department of Biotechnology, University of Agricultural Sciences, Dharwad, India

Madan Bhattacharya Iowa State University, Ames, IA, USA

Subhash Chandra ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Rakeshkumar S. Choudhary Department of Biotechnology, University of Agricultural Sciences, Dharwad, India

Sylvie Cloutier Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada;
Department of Biology, University of Ottawa, Ottawa, ON, Canada

Sandra Cvejić Institute of Field and Vegetable Crops, Novi Sad, Serbia

Inderpreet Dhaliwal Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

Spoorti S. Gandhadmath Department of Biotechnology, University of Agricultural Sciences, Dharwad, India

Sanjay Gupta ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Nada Hladni Institute of Field and Vegetable Crops, Novi Sad, Serbia

Anjana Jajoo School of Life Sciences, Devi Ahilya University, Indore, Madhya Pradesh, India

Chao-Chien Jan Retired, USDA, Agricultural Research Service, Northern Crop Science Laboratory, Edward T. Schafer Agricultural Research Center, Fargo, ND, USA

Siniša Jocić Institute of Field and Vegetable Crops, Novi Sad, Serbia

Milan Jocković Institute of Field and Vegetable Crops, Novi Sad, Serbia

Viraj G. Kamble ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Gurpreet Kaur Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

Jasmeet Kaur Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

Rucha Kavishwar ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Nadeem Khan Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada;
Department of Biology, University of Ottawa, Ottawa, ON, Canada

Virender Kumar National Agriculture Food Technology Institute, Mohali, Punjab, India

Giriraj Kumawat ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India;
Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan

Putta Latha Regional Agricultural Research Station (RARS), Acharya N G Ranga Agricultural University (ANGRAU), Tirupati, India

Yuelian Liu College of Agricultural Sciences, Guangdong Ocean University, Zhanjiang, Guangdong, China

Jiannong Lu College of Agricultural Sciences, Guangdong Ocean University, Zhanjiang, Guangdong, China

Hongmei Miao Henan Sesame Research Center, Henan Academy of Agricultural Sciences, Zhengzhou, China

Dragana Miladinović Institute of Field and Vegetable Crops, Novi Sad, Serbia

Monika ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India

Spurthi N. Nayak Department of Biotechnology, University of Agricultural Sciences, Dharwad, India

Henry T. Nguyen University of Missouri, Columbia, USA

Manish K. Pandey International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India

P. V. V. Prasad Department of Agronomy, Kansas State University, Manhattan, USA

Naveen Puppala Agricultural Science Center at Clovis, New Mexico State University, Clovis, NM, USA

Aleksandra Radanović Institute of Field and Vegetable Crops, Novi Sad, Serbia

S. V. Ramesh ICAR—Central Plantation Crops Research Institute, Kasaragod, Kerala, India

Kusum Rana Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

Milind B. Ratnaparkhe ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Indu Rialch Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

Gyanesh K. Satpute ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Nishtha Shesh ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India;
School of Life Sciences, Devi Ahilya University, Indore, Madhya Pradesh, India

Ajay K. Singh ICAR—National Institute of Abiotic Stress Management, Baramati, Maharashtra, India

Jogendra Singh ICAR—Central Soil Salinity Research Institute, Karnal, Haryana, India

Maharaj Singh ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Naveen Singh ICAR-Indian Institute of Agricultural Research, New Delhi, India

V. V. Singh ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India

Vijayata Singh ICAR—Central Soil Salinity Research Institute, Karnal, Haryana, India

Manoj K. Srivastava ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Palagiri Sudhakar Regional Agricultural Research Station (RARS), Acharya N G Ranga Agricultural University (ANGRAU), Tirupati, India

Sreeja Sudhakaran National Agriculture Food Technology Institute, Mohali, Punjab, India

Rajeev K. Varshney International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India

Donghe Xu Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan

Akwasi Yeboah College of Agricultural Sciences, Guangdong Ocean University, Zhanjiang, Guangdong, China

Xuegui Yin College of Agricultural Sciences, Guangdong Ocean University, Zhanjiang, Guangdong, China

Frank M. You Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada

Jun You Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Haiyang Zhang Henan Sesame Research Center, Henan Academy of Agricultural Sciences, Zhengzhou, China

Xiurong Zhang Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Abbreviations

Δ13C	Carbon isotope discrimination
2-DGE	Two-dimensional gel electrophoresis
A	Cytoplasmic male sterile (CMS) line
ABA	Abscisic acid
ABC	ATP-binding cassette
AFLP	Amplified fragment length polymorphism
AHAS	Acetohydroxy acid synthase
AhTE	*Arachis hypogaea* transposable element
ALS	Acetolactate synthase
AM	Association mapping
AP2	Apetala2 (gene)
APX	Ascorbate peroxidase
AREB	ABA-responsive element binding
AtFTB	*Arabidopsis thaliana* β-subunit of farnesyl transferase
AtGPT1	*Arabidopsis thaliana*-glucose 6-phosphate/phosphate translocator 1
AtIPS2	*Arabidopsis thaliana*-inositol-1-phosphate synthase 2
B	Fertile line
BAC	Bacterial artificial chromosome
BC	Backcross
BC$_1$	First backcross
BC$_1$F$_1$	Testcross
BES	BAC end sequence
bhLH	Basic helix–loop–helix protein
BIM	Bayesian interval mapping
BnCOMT1	*Brassica napus*-caffeic acid O-methyltransferase 1
BnGPT1-C3	*Brassica napus*-glucose 6-phosphate/phosphate translocator 1
BnIPS2-C3	*Brassica napus*-inositol-1-phosphate synthase 2
bZIP	Basic leucine zipper
CaMV35S	*Cauliflower mosaic virus 35S*
CAPS	Cleaved amplified polymorphic sequence

CARG	Core abiotic stress-responsive gene
Cas	CRISPR-associated
CAT	Catalase
CDF	Cation diffusion facilitator
cDNA	Complementary DNA
CG	Candidate gene
CGR	Crop growth rate
Ch	Chromosome
CID	Carbon isotope discrimination
CIM	Composite interval mapping
CMLM	Compressed mixed linear model
CMS	Cytoplasmic male sterility
COMT	Caffeic acid O-methyltransferase
CRIGAAS	Crop Research Institute of Guangdong Academy of Agricultural Sciences
CRISPR	Clustered regularly interspaced short palindromic repeats
CS	Cold stress
CSSL	Chromosome segment substitution lines
CT	Canopy temperature
CTD	Canopy temperature depression
CUP1	Metallothionein gene
CWR	Crop wild relative
DAF	DNA amplification fingerprinting
DArT	Diversity array technology
DEG	Differentially expressed gene
DFIA	Coloration of stigma
DGR	Directorate of Groundnut Research (ICAR, India)
DH	Doubled haploid
DIMS	Direct injection–mass spectrometry
DPPAGSC	Diploid Progenitor Peanut A-Genome Sequencing Consortium
DRE	Dehydration response element
DS	Drought stress
DSI	Drought susceptibility index
DUS	Distinctness uniformity and stability
ECMLM	Enriched compressed mixed linear model
EMMAX	Efficient mixed-model association expedited
EN	Elastic net
ERF	Ethylene-responsive factor
ESI	Electrospray ionization
EST	Expressed sequence tag
F_2	Second filial generation
FAO	Food and Agriculture Organization
FT	Flowering time

FTA	Acclimated freezing tolerance
FTase	Farnesyltransferase
FTB	Acclimation ability
FTN	Nonacclimated freezing tolerance
GA3	Gibberellic acid
GC	Gas chromatography
GCA	General combining ability
GCMS	Gas chromatography–mass spectrometry
GD	Genetic distance
GE	Genome editing
GEBV	Genome-estimated breeding value
GEI	Genotype by environment interaction
GLM	General linear model
GO	Gene ontology
GP	Gene pool
GP	Genomic prediction
GPX	Glutathione peroxidase
GS	Genomic selection
Gs	Stomatal conductance
GSH	Glutathione
GSSG	Glutathione disulfide
GST	Glutathione S-transferase
GWA	Genome-wide association
GWAS	Genome-wide association study/studies
H	Shannon diversity index
HA	Lines for pollination
HDP	Heat denature protein
HI	Harvest index
HIGS	Host-induced gene silencing
HM	Heavy metal
HMA	Heavy metal ATPase
HMM	Hidden Markov Model
HMT	Heavy metal toxicity
HMW	High molecular weight
HPLC	High-performance liquid chromatography
HS	Heat stress
HSP	Heat shock protein
HTS	High-throughput sequencing
IBONE	Instituto de Botánica del Nordeste
ICGR-CAAS	Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IMI	Imidazolinone

InDel	Insertion/deletion
INTA	Instituto Nacional de Tecnología Agropecuaria
IPGI	International Peanut Genome Initiative
IR	Irrigated
IRT	Infrared thermometer
ISSR	Inter-simple sequence repeat
JLAM	Joint linkage association mapping
LAI	Leaf area index
LAS	*Arabidopsis* lateral suppressor
LASSO	Least absolute shrinkage and selection operator
LB	Leaf blotching
LC	Liquid chromatography
LD	Linkage disequilibrium
LEA	Late embryogenesis abundant
LEA4-1	Late embryogenesis abundant 4-1
LF	Leaf firing
LG	Linkage group
LI/LC	Low inoculum with long coculture
LIN	Linolenic acid content
LM	Linear model
LOX	Lipoxygenase
LWI	Leaf wilting index
LWP	Leaf water potential
MAB	Marker-assisted breeding
MABC	Marker-assisted backcross
MABS	Marker-assisted backcrossing
MAGIC	Multiparent advanced generation intercross
MALDI	Matrix-assisted laser desorption ionization
MALDI-TOF	MALDI-time of flight
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MDA	Malondialdehyde
MDHAR	Monodehydroascorbate reductase
MIM	Multiple interval mapping
miRNA	MicroRNA
MLM	Mixed linear model
MRA	Multiple regression association
MRG	Metal-rich granule
mrMLM	Multi-locus random-SNP-effect mixed linear model
MS	Mass spectrometry
MTA	Marker trait association
MTP	Plant metal tolerance protein
MYA	Million years ago
NAM	Nested association mapping

NBPGR	National Bureau of Plant Genetic Resources (India)
NCBI	National Center for Biotechnology Information
ND	North Dakota
NGS	Next-generation sequencing
NILs	Near-isogenic lines
NIR	Non-irrigated
NMR	Nuclear magnetic resonance
NUE	Nutrient use efficiency
OA	Osmotic adjustment
OCRI-CAAS	Oil Crops Research Institute-Chinese Academy of Agricultural Sciences
OIL	Oil content
OP	Open-pollinated varieties
p	Nucleotide diversity parameter
PBR	Plant Breeders' Rights
PCA	Principal component analysis
PCD	Programmed cell death
PCoA	Principal component analysis of molecular marker data
PDX	Ascorbate peroxidase
PGC	Peanut Genome Consortium
PGR	Plant genetic resource
PLC2	Phosphatidyl inositol-specific phospholipase
PM	Powdery mildew resistance
Pn	Photosynthesis
POD	Peroxidase
Pop	Population
PP2C	Protein phosphatases
Pro	Proline
PSS	Percentage of seedlings surviving
PVE	Phenotypic variation explained
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
QTN	Quantitative trait nucleotide
RAPD	Random amplified polymorphic DNA
RBOHD	Respiratory burst oxidase homolog protein D
RD29A	Desiccation-responsive 29A (promoter)
Rf	Fertility restoration gene
RF	Random forest
RFLP	Restriction fragment length polymorphism
RGR	Relative growth rate
RHA-O	Fertility restorer
RILs	Recombinant inbred lines
RKHS	Reproducing kernel Hilbert space
RNAi	RNA interference

RNA-Seq	RNA-sequencing
ROS	Reactive oxygen species
RR	Random regression
RR-BLUP	Ridge regression best linear unbiased prediction
RT-qPCR	Reverse transcription-quantitative PCR
RUBISCO	Ribulose-1, 5-bisphosphate carboxylase/oxygenase
RVI	Relative vigor index
RWC	Relative water content
SAGE	Serial analysis of gene expression
SBP	SQUAMOSA promoter binding protein box gene family
SCA	Specific combining ability
SCMR	SPAD chlorophyll meter reading
SCS	Seed color of stripes
SIM	Simple interval mapping
SLA	Specific leaf area
SMA	Single marker analysis
SMC	Seed main color
SNP	Single-nucleotide polymorphism
SOD	Superoxide dismutase
SRA	Sequence read archive
SS	Salt stress
SSA	Selective sweep analysis
SSD	Single seed descent
SSR	Simple sequence repeat
STI	Stress tolerance index
STS	Sequence-tagged site
SU	Sulfonylureas
SVM	Support vector machine
SVM-RFE	Support vector machine-recursive feature elimination
SWEET	Sugars will eventually be exported transporters
TDM	Total dry matter
TE	Transpiration efficiency
TF	Transcription factor
TILLING	Targeting-induced local lesions in genomes
TL	Transpiration loss
Tr	Transpiration
TRAP	Tartrate-resistant acid phosphatase
U	Usefulness
UPOV	International Union for the Protection of New Varieties of Plants
USDA	United States Department of Agriculture
VIGS	Virus-induced gene silencing
WGRS	Whole genome resequencing
WGS	Whole genome sequencing
WLL	Water loss from excised leaf

WS	Winter survival
WUE	Water use efficiency
Y2H	Yeast two-hybrid system
YAC	Yeast artificial chromosome
YLD	Yield

Chapter 1
Genomic Designing for Abiotic Stress Tolerant Soybean

Milind B. Ratnaparkhe, Gyanesh K. Satpute, Giriraj Kumawat, Subhash Chandra, Viraj G. Kamble, Rucha Kavishwar, Vijayata Singh, Jogendra Singh, Ajay K. Singh, S. V. Ramesh, Virender Kumar, Sreeja Sudhakaran, Manoj K. Srivastava, Nishtha Shesh, Anjana Jajoo, Sanjay Gupta, Maharaj Singh, Donghe Xu, Madan Bhattacharya, and Henry T. Nguyen

Abstract Soybean is an agronomically important crop which is rich in seed protein (about 40%) and oil (about 20%), enriches the soil by fixing nitrogen through symbiosis with bacteria. It is widely used as food, feed, and for industrial purpose. In addition to human consumption, soybean is a major protein source in animal feeds. Soybean is also becoming a major crop for biodiesel production. In soybean, abiotic stresses including drought, temperature extremes, floods, salinity, acidity, mineral

---

M. B. Ratnaparkhe (✉) · G. K. Satpute · G. Kumawat · S. Chandra · V. G. Kamble ·
R. Kavishwar · M. K. Srivastava · N. Shesh · S. Gupta · M. Singh
ICAR—Indian Institute of Soybean Research, Indore, Madhya Pradesh, India
e-mail: milind.ratnaparkhe@icar.gov.in

V. Singh · J. Singh
ICAR—Central Soil Salinity Research Institute, Karnal, Haryana, India
e-mail: Jogendra.Singh@icar.gov.in

A. K. Singh
ICAR—National Institute of Abiotic Stress Management, Baramati, Maharashtra, India

S. V. Ramesh
ICAR—Central Plantation Crops Research Institute, Kasaragod, Kerala, India

V. Kumar · S. Sudhakaran
National Agriculture Food Technology Institute, Mohali, Punjab, India

N. Shesh · A. Jajoo
School of Life Sciences, Devi Ahilya University, Indore, Madhya Pradesh, India

G. Kumawat · D. Xu
Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan
e-mail: xudh@jircas.affrc.go.jp

M. Bhattacharya
Iowa State University, Ames, IA, USA
e-mail: mbhattac@iastate.edu

H. T. Nguyen
University of Missouri, Columbia, USA
e-mail: nguyenhenry@missouri.edu

C. Kole (ed.), *Genomic Designing for Abiotic Stress Resistant Oilseed Crops*,
https://doi.org/10.1007/978-3-030-90044-1_1

toxicity and nutrient deficiency have emerged as the major challenges for achieving the increased productivity. Breeding for tolerance to abiotic stresses is cumbersome due to their genetic control by multiple genes and are also very much influenced by the environment. The novel genomic designing approaches have enabled the improvement of soybean at a faster pace than traditional approaches. Genomic-assisted breeding, genomic selection, genome sequencing, marker-assisted selection, genetic engineering approaches, and genomics tools have been used to improve tolerance to biotic and abiotic stresses, yield and seed composition traits. Genomic designing overcomes the limitations of traditional breeding methods and accelerate the development of climate-smart soybean crops. Developing abiotic stress-tolerant soybean varieties have become convenient with the availability of a complete genomic sequence of soybean and functional genomics studies. This chapter discusses the major milestones in soybean genetics, genome mapping and recent developments in comparative and functional genomics and genome editing related to abiotic stresses.

Keywords Soybean · Abiotic stress · Drought tolerance · GWAS · Functional genomics

1.1 Introduction

1.1.1 Economic Importance of the Crop

Soybean [*Glycine max* (L.) Merr] is an important oilseed crop in the world and serves as a major source of protein and oil for both humans and animals. Soybean forms a raw material for several human health and industrial applications. Besides the edible oil l (18–22%), the seed comprising around 38–45% of protein, and ash, carbohydrate minerals along with antioxidants are major component with potential nutraceutical applications for human health. Hence, soybean has been gaining wide attention in various industries such as food, feed wellness and pharmaceuticals which are attributable to its unique components of minerals, isoflavones, tocopherols etc. Ecologically soybean is involved in biological nitrogen fixation hence improves the soil fertility. Considering its diverse uses the crop is aptly named “miracle bean”. Although the crop is cultivated globally, the United States of America, Brazil, Argentina, China and India are major global producers. Also considering the multiple sectors the crop serves as raw materials, sustainable soybean production is imperative for ensuring global food security.

1.1.2 Reduction in Yield and Quality Due to Abiotic Stresses

Multiple abiotic stressors such as drought, elevated temperature, freezing conditions, floods, soil salinity, acidity and the consequent mineral toxicity or nutrient deficiency are some of the challenges soybean production encounters worldwide. Further, the anticipated frequent extreme in weather conditions due to global climate change is another serious concern for sustainable soybean production. It has been observed that millions of acres of soybean crop loss occur every year due to multiple abiotic factors. Crop loss due to various abiotic stresses demands developing strategies to increase soybean yield or maintain yield stability under multitude of abiotic stresses. Therefore, genomic design of soybean for climate resilience and sustainable production with higher yield potential and nutritional value is mandatory.

1.1.3 Growing Importance in the Face of Climate Change and Increasing Population

The multitude of biochemical characteristics and good quality oil makes soybean a desired oil seed crop and rising its demand worldwide. Nevertheless, the requirement of doubled food production by the end of the year 2050 owing to population explosion will severely squeeze the sufficient production of oil seed crops even more so in the context of changing climatic conditions (Deshmukh et al. 2014). Climate change and extreme weather conditions negatively impact the crop yield while temperature, precipitation, and solar radiation are the main drivers of crop growth and development. Therefore, the breeders are entrusted to provide emphasis on the development of not only high yielding and nutritionally superior soybean genotypes but also the genotypes which are expected to tolerate extreme weather conditions.

1.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

The conventional plant breeding strategies such as single pod descent, backcross breeding, pedigree breeding and bulk population breeding have undoubtedly contributed to the improved soybean yield and tolerance to various abiotic stresses. Nevertheless, these strategies are time consuming and warrant screening of huge plant population that consumes land, labour and water resources. Moreover the breeding for complex traits that are governed by multiple genes are severely influenced by the environment. Further, the complexity of multiple abiotic stresses affecting the standing crop due to climate change have instilled a sense of new urgency into accelerating the rates of genetic gain in molecular breeding programs. Hence, regardless of the conventional breeding efforts, it is imperative to integrate the genome designing based breeding approaches to enhance the production potential of the soybean. To facilitate the advances in soybean breeding, it is indispensable to exploit the molecular breeding techniques such as marker-assisted breeding, recombinant DNA technology, genome editing and multiple “omics” to improve the soybean quality and yield. Hence, these limitations of traditional breeding strategies warrant the large-scale application of genomics science in the improvement of soybean for abiotic stresses.

1.2 Abiotic Stresses and Related Traits in Soybean

1.2.1 Root Characters

Considering the water-deficit stress or flooding stress due to climate change, characteristic features of soybean roots are important to tide over the abiotic stresses. The observed root architecture traits of soybean have revealed that narrow root angle to the soil surface is preferred as it enhances development of lateral roots in the upper root regions where penetration of sunlight is ample. Other root traits such as number of forks, crossings are imperative for good soil penetration, coupled with appreciable root length density (RLD) due to enhanced root surface and root volume. Root characteristic features are important for absorption of soil moisture during stress conditions. Nonetheless deeper soybean roots have not yielded desired results when the soil is shallow or no water at depth or during the conditions of mild water stress (Vadez et al. 2015).

1.2.2 Drought Tolerance

An estimate states that around 40% reduction in soybean production worldwide is due to decrease in water supply and it is also anticipated that such losses would further aggravate due to frequent droughts and water shortages under the scenario of future climate change. Enhancing the irrigation potential is not a viable approach considering the poor resource conditions of the many of the developing countries. This scenario warrants the development of drought-tolerant varieties as an important research urgency. Drought in soybean reduces the economic yield levels by 40% (Specht et al. 1999), however, depending upon the intensity of water-deficit stress and the stage of occurrence, yield losses could be as high as 80%. Phenotyping for drought resistance assumed significance in this context, wherein physiological and biochemical aspects of dehydration avoidance and dehydration tolerance are measured. Breeding for drought tolerance depends on phenotyping methods which are reliable, relatively fast and economical. Generally, the measure of dehydration avoidance involves investigating plant water status, in terms of visual symptoms of leaf senescence, relative water content and analyzing other constitutive traits such as root architecture attributes.

1.2.3 Flooding and Submergence Tolerance

Water logging/flooding is a most deleterious stress next only to drought. Flooding affects the plant health and yield of soybeans in 16% of the soybean production area causes severe economic losses. In US alone flooding stress in soybean causes an annual loss of approximately $1.5 billion (Boyer 1982, 1983; Oosterhuis et al. 1990; Rosenzweig et al. 2002; Bailey-Serres et al. 2012; Ahmed et al. 2013). Flooding stress could be due to submergence or water logging though the former in soybean is a rare occurrence (Oosterhui et al. 1990; VanToai et al. 1994; Linkermer et al. 1998). Water logging or flooding results in reduction in root and shoot growth, decline in atmospheric nitrogen fixation, photosynthetic potential, stomatal conductance and nutrient uptake consequently severely affects the yield of soybean and it may cause death of plant in severe conditions (Sullivan et al. 2001; Shannon et al. 2005; VanToai et al. 2012; Rhine et al. 2012; Wu et al. 2017a).

1.2.4 Heat Tolerance

Yield reduction in soybean due to extreme temperature conditions has been estimated to be around 40% (Specht et al. 1999). Heat stress during vegetative stage affects the growth of soybean. Soybean is highly sensitive to elevated temperature conditions (>35 °C) during reproductive stages as heat stress cause flower and pod abortion during early stages, however the prolonged heat stress during pod filling stages leads to severe reduction in seed size and seed vigour (Boyer 1982; Chebrolu et al. 2016). Therefore, improving heat tolerance of soybean varieties is very crucial to improve the yield levels.

1.2.5 Cold Tolerance

In order to expand the soybean cultivation area from its traditional stronghold it is essential to impart cold tolerance trait so that cultivars could adapt to growing under low temperature conditions. The multiple effects of low temperature on soybean include poor germination, less seedling vigour, flower abortion and poor grain filling at reproductive stages (Yamamoto and Narikawa 1966). Northern hemisphere is characterized with short growing seasons and hence efforts are required to develop soybean varieties having traits such as good emergence and early seedling vigor. Seedling emergence test and early seedling weight are the traits evaluated in soybean germplasm. Genetic dissection of these traits and introgression in cultivated varieties through marker assisted breeding programme is a viable approach to enable the growth of soybean in northern regions.

1.2.6 Salinity Tolerance

Salinity stress severely affects the yields of soybean. High salinity level poses serious damage to the life cycle of soybean whereas low salt levels could cause significant reduction in soybean yield levels (Abel and Mackenzie 1964; Pitman and Läuchli 2002). Various agronomic features of the crop that are affected due to high salinity are significant reduction in plant height, leaf size, biomass, number of pods.plant^{-1}, number of internodes.plant^{-1}, number of branches.plant^{-1}, weight.plant^{-1} and 100 seed weight (Shao et al. 1986; Shao et al. 1993; Parida and Das 2005; Blanco et al. 2007; Bustingorri and Lavado 2011; El-Sabagh et al. 2015). Salt stress observed during the nodulation stage greatly reduces the efficiency of biological nitrogen fixation as severe reduction in number and biomass of root nodules documented (Singleton and Bohlool 1984; Rabie and Kumazawa 1988; Yang and Blanchar 1993; Delgado et al. 1994; Elsheikh and Wood 1995). Soybean germplasm display a spectrum of salt tolerance capability (Yang et al. 1993; Pitman and Läuchli 2002; Lenis et al. 2011).

1.3 Genetic Resources of Resistance/Tolerance Genes

The diverse morphological, cytological and genetical features of wild species of soybean and also the cultivated soybean display wide array of genetic sources of resistance to multiple biotic and abiotic stresses. Thus, wild species form an important component of gene pool for the exploration of useful genes and alleles conferring abiotic stress tolerance. The annual and perennial soybean species are significantly distantly related. Wild perennial *Glycine* species offer immense potential for soybean improvement. The genus *Glycine* has two subgenera, *Glycine* Willd. (perennial) and *Soja* (Moench) F.J. Herm (annual). The subgenus *Soja* includes two species: the cultivated soybean [(*G. max* (L.) Merr.)] and its wild annual progenitor *G. soja* Sieb. & Zucc. (Ratnaparkhe et al. 2010). The subgenus *Glycine* comprises 30 wild perennial species thus, the genetic resources of soybean may be categorized into four plausible gene pools (GP).

1.3.1 Soybean GP-1

Soybean gene pool -1 (GP-1) comprise all of the biological species which could be crossed among them to yield vigorous hybrids characterized with normal meiotic chromosome pairing and possess total seed fertility. All soybean (*G. max*) germplasm and the wild soybean, *G. soja*, constitute GP-1 with the qualification that seed sterility can be associated with chromosomal structural changes such as inversions and translocations. Gene segregation is normal and gene exchange is generally easy.

1.3.2 Soybean GP-2

GP-2 include those species which can hybridize with GP-1 with relative ease and the resultant F_1 plants exhibit at least some seed fertility (Harlan and de Wet 1971). *Glycine max* is devoid of GP-2 because no known species exhibit such a relationship with soybean. Though it is plausible for existence of such species in Southeast Asia where the *Glycine* genus may have originated, extensive germplasm exploration is indispensable to validate this suggestion.

1.3.3 Soybean GP-3

GP-3 is a potential genetic resource of soybean even though the hybrids between GP-1 and GP-3 are lethal. Gene transfers between GP-1 and GP-3 are not possible without resorting to in vitro culture based techniques such as embryo rescue etc. (Harlan and de Wet 1971). GP-3 includes the 26 wild perennial species of the subgenus *Glycine* which are indigenous to Australia and remain geographically isolated from *G. max* and *G. soja.* The three species *G. argyrea*, *G. canescens*, and *G. tomentella* have been successfully hybridized with soybean embryo culture based rescue techniques ensured the survival of F_1 hybrids. However, much progress has not been made beyond the amphidiploid stage, with the exception of Singh et al. (1998a, b) suggesting that only these three species belong to GP-3.

1.3.4 Soybean GP-4

GP-4 is considered an extremely outer limit of genetic resources of soybean. Several pre- and post-hybridization barriers arrests the process of embryo development resulting in premature abortion of embryo. Bridge crosses with genus *Glycine* could circumvent the problems of seedling lethality, seed inviability and inviable F1 plants (Singh et al. 2007). Hence restricting the utility of GP-4 only few wild perennial *Glycine* species have been hybridized with soybean. Thus, majority of species belong to soybean GP-4 as they have not been hybridized with GP-1 when hybridized did not produce viable F_1 plants (Singh et al. 1987). Although the wild perennial species exhibit resistance to several biotic and abiotic stresses, the transfer of useful genes into soybean has not been accomplished. Thus, the breeders/geneticists have access to the primary gene pool for expanding the germplasm base.

1.4 Classical Genetics and Traditional Breeding of Abiotic Stress Tolerance in Soybean

1.4.1 Classical Mapping Efforts

Soybean has been under continuous investigation for its genetic improvement by plant breeders. The crop encounters various biotic and abiotic stresses and hence improving their tolerance to stresses along with seed composition traits is pertinent. Improving agronomic performance of the crop would ensure higher productivity, and improved consumption of soybean and soy products leading to realization of greater economic benefits. Plant breeders have been traditionally using the practices of hybridization and meticulous selection methods to ensure better performing genotypes resulting in development of many soybean varieties. Classical genetics and traditional breeding approaches have been used to develop tolerance for drought, waterlogging stress, salt tolerance and for other abiotic stresses. Table 1.1 lists the soybean lines and resources used for the genetic improvement of abiotic stress tolerance.

1.4.2 Breeding Objectives

Designing highly productivity genotypes under water-limited conditions is an important breeding objective. It warrants introgression of physiological traits that define plant water relations and hydraulic processes, into a common genetic background (Satpute et al. 2021). Water deficit condition is an outcome of complex interplay of several factors including low soil moisture and extreme temperatures and other edaphic factors. Breeding promising soybean genotypes through transfer of gene(s) conferring drought tolerance is an effective approach to alleviating the ill-effects of drought. Under drought stress, soybean plants suffer from oxidative injury, membrane system damage, cellular ion leakage and protein denaturation, declined photosynthetic rates, and CO_2 uptake rates consequently causing reduction in biomass accumulation and yield levels. Hence, under drought, among the various physiological processes, photosynthesis is severely down-regulated with wider ramifications for the economic yield levels of the crops.

Breeding approaches to develop drought tolerant soybean involve diverse strategies namely recurrent selection and evaluation of segregating population under imposed drought-stress environment, and investigating the secondary traits for efficient selection, molecular breeding for drought tolerance, genomics-based and transgenic technologies to improve the drought tolerance trait. Advance phenotyping-based breeding approaches are pre-requisite and are being adopted systematically by developing early generation biparental, backcross or multi-parent intercross populations using identified candidate drought tolerant parental lines and wider-adaptable high yielding varieties. The populations are advanced through F_2 generation by mass selection where bulks are subjected to chemical desiccation process using potassium

Table 1.1 Potential genetic resources for abiotic stress tolerance

Tolerant genetic resource/genotype	Source	References
Drought tolerance		
PI 416,937	USDA, ARS	Sloane et al. (1990), Patterson and Hudak (1996), Sinclair et al. (2007), King et al. (2009)
Young	USDA, ARS	Mian et al. (1996)
Jackson	–	Purcell et al. (1997)
PI 407,155	–	Chen et al. (2006)
R01-416F and R01-581F	USA	Chen et al. (2007)
93,705-36 and PI471938	USDA	King et al. (2009)
PI 468,917	–	Seversike et al. (2014)
C12 and W05	CUHK, China	Hossain et al. (2014)
PI 567,690 and PI 567,731	China	Pathan et al. (2014)
EC 538,828, JS 97–52 and EC 602,288	India	Bhatia et al. (2014), Bhatia and Jumrani (2016)
NTCPR94-5157, N09-13,890, NC-Raleigh, and SC07-1518RR	USA	Fried et al. (2019)
PK 1180 and SL 46	India	Sreenivasa et al. (2020)
TGX 709-7E and EC 389,174	India	Satpute et al. (2020a)
Water logging tolerance		
Edison, GR 8836, CX 415	USA	VanToai et al. (1994)
Archer	USA	VanToai et al. (2001)
PI 408105A	Korea	Shannon et al. (2005)
Misuzudaizu	Japan	Githiri et al. (2006)
91,210–350, 91,210–316	USA	Henshaw et al. (2007)
Kefeng No. 1	China	Wang et al. (2008b)
Peking	China	Sayama et al. (2009)
Nam Vang, VND2, ATF15-1	Cambodia	VanToai et al. (2010)
AGS 313	Bangladesh	Ara et al. (2015)
JS 97–52 and JS 20–38	India	Anonymous (2015)
Iyodaizu	Japan	Nguyen et al. (2017)
Kokubu 7, Maetsuezairai 90B, Yahagi	Japan	Suematsu et al. (2017)

(continued)

Table 1.1 (continued)

Tolerant genetic resource/genotype	Source	References
UA 5615C, R10-4892, R13-12552, R07-6669, Walters, R04-342, S11-25,108, S12-1362, S11-25,615	USA	Wu et al. (2017a)
JS 20–38, C 2797, Hardee, IS 128 and JS 71–05	India	Chandra et al. (2020)
PI 561,271, PI 567,651, PI 567,343, PI 407,184,PI 603910C, PI 567394B, PI 467,162, PI 479,751, PI 407,229, PI 597459C, PI 424,082, PI 378699A, PI 424107A, PI 366,124	USA	Valliyodan et al. (2017)
Salt tolerance		
S-100, Centennial, Cook, D49-2491, Dillon, Forrest, Gordon, Haskell, Hill, Hutton, Johnston, Lee, Manokin, Wright	**USA**	Lee et al. (2004)
Nannong 1138–2	China	Chen et al. (2008)
Jindou no. 6 (PI574484)	China	Hamwieh et al. (2011)
FT-Abyara (PI628838)	Brazil	Hamwieh et al. (2011)
JWS156-1	Japan	Hamwieh and Xu (2008)
PI483463	China	Lee et al. (2009)
Tiefeng 8	China	Guan et al. (2014a)
W05	China	Qi et al. (2014)
Fiskeby III	Sweden	Do et al. (2018)
Osage	USA	Zeng et al. (2017)
Jidou 12	China	Shi et al. (2018)
SL 1226, SL 1258	India	Singh et al. (2020)
PI597458B, PI342434, PI548198, PI561389B, PI407202, PI407220, PI424107A, PI479752, PI407083, PI468908, PI080837, PI417500, PI424116, PI483460B, PI562551	USDA Soybean Germplasm Collection	Do et al. (2019)

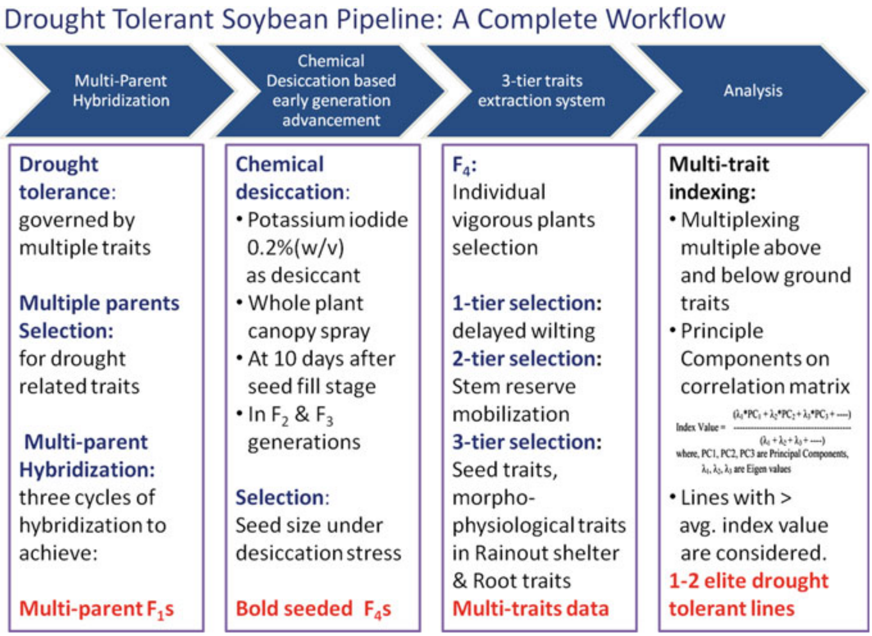

Fig. 1.1 Workflow for developing drought tolerant parental lines and varieties specially for target population of environments (TPE) of drought conditions occurring at seed filling growth stage

iodide 0.2% (Blum et al. 1991; Bhatia et al. 2014; Satpute et al. 2019) followed by selections. Mass selection for the trait large seeds following chemical desiccation has significantly improved the seed weight and grain yield under chemical desiccation stress compared to control set up wherein seed selection was performed without chemical desiccation (Blum 2011). After two cycles of selection, intensive investigation of candidate genotypes for multiple drought tolerance-related traits is practiced in advance generations using three-tier selection scheme followed by multi-traits indexing. Figure 1.1 shows the scheme involved in developing drought tolerant soybean (Satpute et al. 2021). Development of soybean varieties with enhanced tolerance to drought, heat, salinity and cold has become a high research priority in major breeding programs worldwide (Fig. 1.2).

1.5 Genetic Diversity Analysis

1.5.1 Phenotype and Genotype Based Diversity Analysis

During the past three decades, genetic diversity studies in soybean has been dominated by phenotyping-based diversity analysis, cytogenetics and molecular studies,

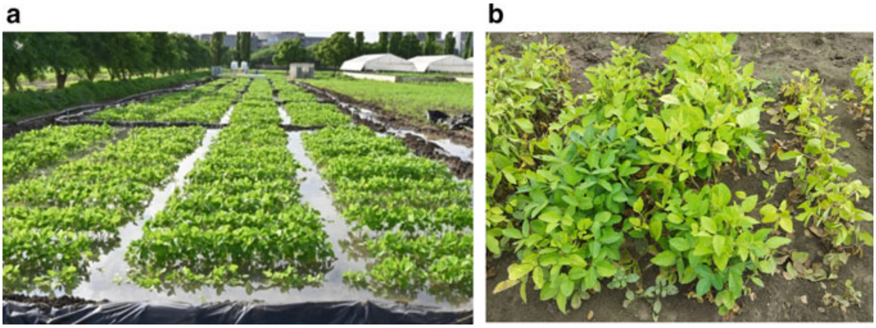

Fig. 1.2 **a** and **b** Screening of soybean germplasm under field condition for tolerance to water logging

including variation in isozymes and, seed proteins, use of restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA(RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers. The geographic differentiation in Chinese cultivated soybean and genetic diversity was studied using the coefficient of parentage (Cui et al. 2000a), morphological traits (Dong et al. 2004), SSR markers (Li et al. 2008a; Wang et al. 2008a; Li et al. 2010; Wang et al. 2015) and SNP markers (Kajiya-Kanegae et al. 2021; Saleem et al. 2021). The diversity analysis of Asian soybean landraces and North American cultivars revealed a low level of diversity in the American pools than in the Asian pools, based on phenotypic characterization (Cui et al. 2000a, 2001) or the coefficient of parentage analysis (Cui et al. 2000b). Low diversity was further confirmed in DNA sequence-based analyses showing successive genetic bottlenecks between wild and cultivated soybeans and between Asian landraces and North American cultivars (Hyten et al. 2006). Genetic diversity studies in soybean have been discussed in detail by Carter et al. (2004). Comparison of Chinese and American Soybean Accessions using High-Density SNPs in population structure analysis, and cluster analysis revealed that the genetic basis of Chinese soybeans is entirely distinct from that of the USA (Liu et al. 2017).

1.5.2 Relationship with Other Cultivated Species and Wild Relatives

Comprehensive biosystematics based relationship analysis of all species in the genus *Glycine* reveal that annual (subgenus *Soja*) and perennial (subgenus *Glycine*) soybean species are significantly distantly related (Doyle et al. 2003), having diverged from a common ancestor around 5 MYA (Innes et al. 2008). As stated above in genetic resources section, several attempts to hybridize species between the subgenus *Soja* and subgenus *Glycine* were unsuccessful. The pods resulted from interspecific hybridization eventually aborted and got abscised although pod development was found to be initiated (Ladizinsky et al. 1979a; b). Later, the intersubgeneric F_1 hybrids of *G. max* × *G. clandestina*, *G. max* × *G. tomentella* and *G. max* × *G. canescens* were successfully obtained *either following* embryo rescue technique (Newell and Hymowitz 1982; Singh and Hymowitz 1985; Singh et al. 1987) or using transplanted endosperm as a nurse layer (Broué et al. 1982). In general, the cultivated soybean could only hybridize with members of the subgenus *Glycine* imperfectly. The progeny of such inter-subgeneric hybrids were completely sterile and obtained with a great difficulty. Studies have proven that cultivated soybean does not hybridize with any of the wild relatives in other genera of the tribe (Hymowitz et al. 1995; Hymowitz 2004). The wild soybean (*G. soja*) has accumulated rich genetic diversity in the process of evolution and adaptation (Kofsky et al. 2018). This adaptive evolutionary process has resulted in wide diversification in the traits of wild soybean. The diversity for multiple morphological features includes flower, pubescence, seed and hilum color, disease and insect resistance traits, physiological and biochemical traits (protein, oil and carbohydrates and their constituents content) (Boerma and Specht 2004).

1.6 Association Mapping Studies

1.6.1 Extent of Linkage Disequilibrium

Linkage disequilibrium (LD) describes changes in the genetic variation within a population over time. Variation in LD either at genome scale or at a particular-genomic region is influenced by various factors such as mutation, domestication, level of inbreeding and selection, confounding effects, population admixture, and population substructure (Rafalski and Morgante 2004). A strong correlation is anticipated between inter-locus distance and LD if the recombination rates do not vary across the genome particularly in a constant population size. Soybean, owing to its ineffective recombination and homozygous genetic background, exhibit less decay of LD (longer region is in LD).

1.6.2 Genome-Wide LD Studies

SNPs are choice markers due to its abundant DNA polymorphism and hence are useful in genetic diversity studies and in determination of genetic relatedness among the individuals. To investigate the genetic frequency of SNP in soybean genome, ~28.7 kbp of coding region, 37.9 kbp of non-coding perigenic region, and 9.7 kbp of random non-coding genomic regions were evaluated in 25 diverse soybean genotypes (Zhu et al. 2003). This study divulged that the nucleotide diversity (θ) observed in coding and in non-coding perigenic DNA was 0.00053 and 0.00111, respectively.

The combined nucleotide diversity of whole sequence was 0.00097. Squared allele frequency correlations (r^2) among haplotypes at 54 loci with two or more SNPs indicated low genome-wide LD. A haplotype map of soybean (GmHapMap) was constructed using whole-genome sequence data from 1007 *Glycine max* accessions yielding 14.9 million variants as well as 4.3 M tag SNPs (Torkamanhe et al. 2021). A lower level of genome wide genetic diversity was observed in soybean compared to other major crops. Genome-wide LD investigations in soybean have facilitated identification of molecular markers and key genes associated with various abiotic stresses.

1.6.3 Genome Wide LD Studies for Drought Tolerance

Quantitative trait locus (QTL) mapping using bi-parental populations is limitated by restricted allelic diversity of parental genotypes and mapping resolution. The allelic diversity among mapping populations can be increased to some extent by using multi-parental crosses (Deshmukh et al. 2014). The genome wide association study (GWAS) approach provides opportunities to explore the tremendous allelic diversity present in soybean germplasm. Since millions of crossing events and natural mutations have been fixed in the germplasm during evolution, the mapping resolution of GWAS is comparatively higher. The recent advances in sequencing have played an important role in performing the genome- wide association studies (Abdurakhmonov and Abdukarimov 2008). GWAS is now routinely being used in soybean and other plant species, however fewer studies have been reported with regards to different abiotic stresses. GWAS for quantitative traits like drought tolerance are predicted to be affected by population structure. GWAS models like mixed linear model (MLM) and compressed mixed linear model (CMLM) have been developed which takes into account the population structure, kinship and spurious allelic associations (Deshmukh et al. 2014). Recent development in statistical tools involving larger set of genotypes and high throughput genotyping approaches will definitely improve GWAS power.

Dhanpal et al. (2015) analyzed a population of 373 genotypes in four environments for carbon isotope ratio (δ13C), an important physiological trait linked with water use efficiency (WUE). An association of 39 SNPs, linked to 21 different loci involved in conferring drought tolerance trait has been found. Similarly, Kaler et al. (2017) reported 54 SNPs associated with δ^{13}C & 47 SNPs associated with δ^{18}O. These SNPs were tagged with 46 putative loci and 21 putative genetic loci for δ^{13}C and δ^{18}O, respectively. Several markers and loci have been reported for various drought related traits viz., chlorophyll fluorescence (Hao et al. 2012; Herritt et al. 2018), canopy temperature (Kaler et al. 2017), delayed canopy wilting (Steketee et al. (2020) and drought susceptibility index (Chen et al. 2020) (Table 1.2). GWAS analysis in soybean for drought tolerance was reported using 259 Chinese cultivars for drought related traits. This investigation was based on a total of 4,616 SNPs, and 15 SNP-trait

Table 1.2 Details of genome wide association studies (GWAS) carried out for abiotic stress tolerance

Trait	GWAS loci	Markers	Genotypes	Method	References
Drought tolerance					
Chlorophyll and chlorophyll fluorescence parameters	51	1536 SNP	168	MLM	Hao et al. (2012)
Ureide concentration	53	33,957 SNP	374	PROC GLIMMIX	Roy et al. (2014)
Carbon isotope ratio (δ13C)	21	12,347 SNP	373	GLM & MLM	Dhanpal et al. (2015)
Chlorophyll contents	27	31,253 SNP	332	MLM	Dhanpal et al. (2016)
Carbon isotope ratio (δ13C) Oxygen isotope ratio (δ13C)	46 21	31,260 SNP	373	Farm-CPU	Kaler et al. (2017)
Canopy temperature	34	31,260 SNP	345	Farm-CPU	Kaler et al. (2017)
Chlorophyll fluorescence	53	32,453 SNP	189	CMLM	Herritt et al. (2018)
Delayed canopy wilting	44	34,379 SNP	162	MLM	Steketee et al. (2020)
Germination under drought	15	4,616 SNP	259	MLM	Liu et al. (2020a)
Drought susceptibility index and yield traits	302	105,970 SNP	136	MLM	Chen et al. (2020)
Water logging tolerance					
Seed-flooding tolerance in terms of germination rate, normal seedling rate and electric conductivity	25	60,109 SNPs	347	MLM and mrMLM	Yu et al. (2019)
Foliar damage score	14	31,125 SNPs	384	GLM, MLM, CMLM., ECMLM	Wu et al. (2020)
Salinity tolerance					
Seed germination under salt stress	8	1142	191	MLM	Kan et al. (2015)

(continued)

Table 1.2 (continued)

Trait	GWAS loci	Markers	Genotypes	Method	References
Leaf chloride concentrations and leaf chlorophyll concentrations	45	33,009	283	GLM, MLM	Zeng et al. (2017)
Leaf scorch score	62	37,281	192	GLM, MLM	Huang et al. (2018)
leaf scorching score (LSS), chlorophyll content ratio(CCR), leaf sodium content(LSC) and leaf chloride content (LCC)	8	42,000	305	EMMAX, MLMM	Do et al. (2019)
LSS, CCR, LSC and LCC	29	3.7 M	305	EMMAX, MLMM	Do et al. (2019)
Salt tolerance at germination stage	18	207,608	211	CMLM	Zhang et al. (2019)

associations were identified by GWAS, among which three SNPs were suggestively associated with two of the drought-tolerance indices (Liu et al. 2020a).

1.6.4 Genome-Wide Association Mapping for Flooding Tolerance

Genome-wide association mapping has advantages over bi-parental QTL mapping as the former exploits the historical and evolutionary recombination (Zhu et al. 2008). Yu et al. (2020) conducted GWAS in a panel of 347 soybean genotypes to identify SNPs associated with seed-flooding tolerance related traits, viz., germination rate (GR), normal seedling rate (NSR) and electrical conductivity (EC). Use of 60,109 SNPs identified three major QTNs, viz., QTN13, qNSR-10 and qEC-7–2 linked to the traits. Further, QTN13 was consistently identified in all three traits investigated and in multiple environments. Wu et al. (2020a) applied GWAS in a panel of 384 soybean lines, using 42,291 SNP markers and models viz. regression linear model (GLM), mixed linear model (MLM), compressed mixed linear model (CMLM), and enriched compressed mixed linear model (ECMLM) for dissecting flooding tolerance. It has resulted in identification of 14 SNPs associated with flooding tolerance across all the environments and models (Table 1.2).

1.6.5 Genome-Wide Association Mapping for Salt Tolerance

Seed germination under salt stress was used for an association mapping study by Kan et al. (2015). Under salt stress, three loci significantly associated with three traits namely the ratio of imbibition rate, the ratio of germination index, and the ratio of germination rate, were identified and mapped to chromosomes Gm08, Gm09 and Gm18. Using 283 diverse lines of worldwide soybean accessions, In another GWAS study, Zeng et al. (2017a) identified eight genetic loci (mapped on to chromosomes Gm02, Gm7, Gm08, Gm10, Gm13, Gm14, Gm16, and Gm20) associated with leaf chloride and leaf chlorophyll concentrations by using sing 283 diverse lines of soybean accessions. Huang et al. (2018) used a diverse set of 192 soybean germplasm and identified six genomic regions (Gm02, Gm03, Gm05, Gm06, Gm08 and Gm18) associated with salt tolerance based on visual leaf scorch score. The study by Do et al. (2019), using two GWAS populations for association mapping of salt tolerance, confirmed the major locus on chromosome Gm03 and identified three novel loci on Gm01, Gm08 and Gm18. Several SNPs have been identified to be significantly associated with traits, leaf scorching score (LSS), chlorophyll content ratio (CCR), leaf sodium content (LSC) and leaf chloride content (LCC) (Do et al. 2019). Zhang et al. (2019) identified genomic regions associated with salt tolerance at germination stage and showed 18 significant SNPs were located on chromosome Gm08 and Gm18. Seventeen of the 18 significant SNPs were located in a major QTL*qST-8*, which was identified by linkage mapping in recombinant inbred lines (RILs) (Zhang et al. 2019). Though GWAS studies for salinity stress are relatively few in soybean, besides confirming major genetic determined by linkage mapping, GWAS has provided information of tolerant loci from new germplasm sources, which are quite useful in QTL pyramiding (Table 1.2).

1.7 Molecular Mapping of Tolerance Genes and QTLs

1.7.1 Brief History of Molecular Mapping in Soybean

The first report of utilization of molecular markers in soybean is use of restriction fragment length polymorphism (RFLP) for the assessment of genetic diversity of the soybean nuclear genome (Apuya et al. 1988). Subsequently, RFLP markers were used extensively for genetic diversity analysis (Kiem et al. 1989; Skorupska et al. 1993; Lorenzen et al. 1995) and linkage mapping (Kiem et al. 1990; Diers et al. 1992; Lark et al. 1993; Akkaya et al. 1995; Shoemaker and Specht 1995; Mansur et al. 1996; Kiem et al. 1997; Cregan et al. 1999; Ferreira et al. 2000; Yamanaka et al. 2001; Lightfoot et al. 2005) until SSR and SNP markers become popular (Hyten et al. 2010a), Lee et al. (2015), Sun et al. (2019a), Ratnaparkhe et al. (2020), Kumawat et al. 2021).

1.7.2 *Evolution of Marker Types and Genetic Diversity Studies*

Various marker-based technologies such as RFLPs, RAPDs, AFLPs, SSRs and SNPs were used for genetic mapping and diversity studies in soybean. Apuya et al. (1988) analyzed randomly chosen 300 RFLP probes in genomic DNA of the genetically distant cultivars Minosy and Noir 1. RAPDs were also used extensively by soybean geneticists, mainly for germplasm classification (Thompson et al. 1998; Brown–Guedira et al. 2000; Li and Nelson 2002). Construction of soybean linkage maps was done using SSR and AFLP markers (Morgante et al. 1994; Keim et al. 1997; Matthews et al. 2001). Interestingly, the first report of SSR allelic variation and their use as marker system in plant species was in soybean (Akkaya et al. 1992; Morgante and Oliveri 1993). SSR polymorphism showed high level of allelic variation in cultivated and wild soybean genotypes (Morgante et al. 1994; Maughan et al. 1995; Rongwen et al. 1995; Li et al. 2010). Akkaya et al. (1995) developed SSRs in soybean and integrated them into the linkage map. Subsequently, Cregan et al. (1999) mapped 606 SSR loci to develop an integrated soybean linkage map which was subsequently improved by addition of 420 SSRs (Song et al. 2004; Cregan et al. 1999). Hisano et al. (2007) used EST sequences to map a total 668 EST-derived SSR marker loci on soybean linkage map. Further, the availability of BAC-end sequence facilitated development of additional SSRs leading to integration of physical map and genetic map (Shultz et al. 2007; Shoemaker et al. 2006). Utilizing the whole genome sequence of soybean, a SSR database (BARCSOYSSR_1.0) was developed by Song et al. (2010). This genome-wide SSR database provides informative SSRs at any genomic position required for fine mapping as well as for MAS. Choi et al. (2007) identified SNPs via the resequencing of sequence-tagged sites (STSs) developed from EST sequences. In the total 2.44 Mbp of aligned sequence, a total of 5,551 SNPs were discovered, including 4712 single-base changes and 839 InDels resulting in an average nucleotide diversity of $\theta = 0.000997$. Exploiting these SNPs, a total of 1,141 genes were placed on the genetic map by virtue of a SNP segregating among one or more RIL mapping populations, thus constructed a transcript map in soybean. Recent advances in whole genome sequencing and high throughput genotyping helped in the large scale genetic diversity studies of soybean germplasm collections.

1.7.3 *Mapping Populations*

Various mapping populations in soybean have been developed independently based upon the interests and needs of individual researchers, i.e., the degree of polymorphism required and specific agronomic traits for analysis. F_2 populations or recombinant inbred lines (RILs) have been employed for the construction of linkage maps in soybean. While interspecific mapping populations have contributed enormously to the saturation of the soybean linkage map, intraspecific linkage maps have also been developed. Recently, Nested association mapping (NAM) have been used for genetic mapping in soybean (Diers et al. 2018; Beche et al. 2020).

1.7.4 QTL Mapping Studies

Molecular markers especially DNA-based markers have been used extensively to identify the genomic locations of major QTLs governing different traits in soybean. RILs which are developed following several generations of selfing (typically up to F_6 or F_7) are used in mapping QTLs. RILs are helpful in dissecting the QTLs and the estimate of influence of single or few QTL is possible depending on the population size. More than thousand QTLs governing over 100 agronomically and physiologically important traits have been characterized or mapped in soybean (Grant et al. 2010). Information pertaining to the QTLs mapped in soybean is available on database *SoyBase* (http://soybase.org). Recently, the advent of SNP-based genetic markers has facilitated the QTL analysis of many agronomic traits of soybean (https://soybase.org, http://soykb.org). The developments in the field of whole genome sequencing and the popularity of high throughput technologies have facilitated the genetic mapping in soybean in a great way yielding millions of SNP markers (Schmutz et al. 2010).

QTL mapping and molecular marker development have advanced in dissecting several agronomic traits and in studying the genetic basis of resistance against drought and water logging along with improved yield. In the pursuit to develop genotyping tools for investigating mapping population, Hyten et al. (2008) has developed a multiplex assay designated as soybean oligo pool all-1 (SoyOPA-1). This custom-made 384-SNP GoldenGate assay was developed utilizing SNPs discovered through resequencing of five diverse soybean accessions. Later, Hyten et al. (2010a) sequenced a total of 3,268 SNP-containing robust STS in six diverse genotypes, resulting in identification of 13,042 SNPs with an average of 3.5 SNP per polymorphic STS. These SNPs along with 5,551 SNPs discovered by Choi et al. (2007) were used to design two Illumina custom 1536 SNP GoldenGate assays designated as SoyOPA-2 and SoyOPA-3. A set of 1,536 SNPs (from the 3456 SNPs in three SoyOPAs) designated as Universal Soy Linkage Panel 1.0 (USLP1.0), ensured sufficient polymorphic markers at genome scale for use in QTL mapping applications. Hyten et al. (2010b) sequenced a reduced representation library of soybean to identify SNPs using high throughput sequencing methods. A total of 1,536 SNPs were selected to create an Illumina GoldenGate assay (SoyOPA-4). The SoyOPA-4 produced 1,254 successful GoldenGate assays suggesting an assay conversion rate of 81.6% for the predicted SNPs. Chaisan et al. (2010) used ESTs derived from 18 genotypes for EST clustering and SNP identification resulting in a total of 3,219 EST contigs and a total of 26,735 SNPs. The confirmation of in silico identified SNPs by Sanger sequencing yielded 15.7% accuracy rate between two cultivars Williams 82 and Harosoy. SNP markers in soybean which could be utilized for mapping of complex traits as well as molecular breeding applications have been developed in recent investigations (Song et al. 2012; Li et al. 2019; Song et al. 2020).

1.7.5 QTL Mapping Software

QTL mapping in soybean has progressed swiftly in last three decades or so nonetheless, a large fraction of QTLs remains unutilized in breeding programs because of issues such as low accuracy and false-positives. However, the QTL accuracy could be improved by adopting various QTL mapping methods and effective statistical models such as single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM), multiple interval mapping (MIM), and Bayesian interval mapping (BIM). Various QTL mapping softwares and QTL network have been developed to perform the task. "Meta-QTL analysis" compile QTL data from multiple reports onto a same map to ensure precise identification of QTL regions (Deshmukh et al. 2012; Sosnowski et al. 2012). Meta-QTL was effectively utilized by Hwang et al. (2015) to identify QTLs linked to Canopy wilting using l five different populations (RILs). Among the QTLs identified, one QTL on chromosome 8 in the 93,705 KS4895 × Jackson population co-segregated with already known QTL linked with wilting identified in a Kefeng1 × Nannong 1138–2 population. The advances in statistical approaches and software resulted in exponential increase in soybean genetic mapping studies to understand plants response to extreme climatic conditions for abiotic stress such as drought, water logging and high temperature stress.

1.8 Marker-Assisted Breeding for Resistance/Tolerance Traits

Marker-assisted selection (MAS) is an indirect selection method where the linked molecular marker is utilized to transfer important agronomic traits from one genotype to another genotype. Marker-assisted backcrossing (MABC) is an important approach employed in soybean for transferring trait of interest. The high-throughput genotyping technologies have greatly assisted in the process of molecular marker identification and QTL mapping for different traits in soybean. The molecular breeding approaches such as Marker-assisted backcrossing and marker-assisted recurrent selection have aided in the introgression of the trait of economic or agronomic interest in soybean. In the past decades, several studies have focused on the genetic and molecular mechanisms of drought tolerance, flooding tolerance, salt tolerance where several QTLs have been identified to be associated with various abiotic stresses.

1.8.1 QTL Mapping for Drought Tolerance

Drought tolerance is a complex trait influenced by multiple genetic locations or governed by polygenes/QTLs, introgression of minor QTLs from donor to recipient cultivar is not an easy task. QTL mapping identified a total of 10 genomic regions associated with canopy wilting under drought stress (Table 1.3). Majority of these QTLs (9/10) have donor alleles conferring slow wilting traits from PI 416,937, Jackson, or both (Charlson et al. 2009; Abdel-Haleem et al. 2012; Hwang et al. 2015). Molecular markers associated with these QTLs could be explored for use in MAS to introgress the slow canopy wilting phenotypes from the donor into the elite backgrounds. However, transferring these QTLs is challenging task owing to the comple and, quantitative nature of the trait along with its sensitivity to prevailing environmental factors. Most minor QTLs were found to be unstable across the environments and populations. For instance, even major QTLs on chromosome 12 ($R2 =$ 0.27) identified in all five environments from Benning × PI 416,937 (Abdel-Haleem et al. 2012) was not detected in any populations or site-years (Hwang et al. 2015). Accordingly, it is mandatory for QTL confirmation in more advanced generations to validate each individual QTL. It also suggests that molecular stacking of all confirmed QTLs in the genetic background of an elite cultivar is imperative to develop drought tolerance in soybean (Valliyodan et al. 2016). Ren et al. (2020) identified 23 QTL linked to drought tolerance of which seven QTLs were identified on chromosomes 2, 6, 7, 17, and 19 while five QTL were found on chromosomes 2, 6, 13, 17, and 19 respectively.

1.8.2 QTL Mapping for Root System Architecture and Canopy Characteristics

Mapping of genomic regions controlling root system architecture (RSA) and canopy characteristics is critical to develop soybean that is cultivable in water-limited environment (Song et al. 2016a). In an interspecific RIL population derived from cross *G. max* (V71-370) × *G. soja* (PI407162), four significant QTLs associated with different root architectural traits were identified on chromosome Gm06 and Gm 07 (Prince et al. 2015a). In another study, Manavalan et al. (2015) identified a major QTL on chromosome Gm08 controlling tap root length, lateral root number and shoot length. Six transcription factors and two key cell wall expansion-related genes were identified as candidate genes in the confidence interval of this QTL. Recently, Dhanpal et al. (2021) conducted first genome-wide association study reporting genetic loci for RSA traits for field-grown soybean and identified key candidate genes.

Table 1.3 Overview of QTLs identified for abiotic stress (Drought, water logging) tolerance in soybean depicting parents, mapping population, associated trait, chromosome and markers and phenotypic variance explained

Parents involved	Population/Size	Traits related to drought tolerance	Linked marker(s)	Type of marker	LG/Ch No	QTLs/genes	PVE(%)	References
Drought tolerance								
Young × PI416937	F_4 (120)	Water use efficiency, leaf ash	cr497-1	RFLP	J	3 QTLs	13.2	Mian et al. (1996)
S100’ × “Tokyo,”	F_2 (116)	Water use efficiency	A489H	RFLP	L	2 QTLs	14	Mian et al. (1998)
Minsoy’ × Noir 1’	RIL (236)	Transpiration efficiency, yield and carbon isotope discrimination	Satt205, satt489	SSR	C2	3 QTLs	7–12.8	Specht et al. (2001)
Kefeng 1 × Nannong 1138-2	RIL	Dry root weight, total root length and root volume, canopy-wilting trait	–	–	N6-C2 N8-D1b + W N11-E, N18-K	13 QTLs	–	Liu et al. (2005)
Jackson × KS4895	RIL (81)	Leaf wilting	Sat_044	SSR	K	1 QTL	17	Bhatnagar et al. (2005)
Hutcheson × PI471938	F_4 (140)	Yield and wilting	Satt226, Sat_375, Sat_074	SSR	D2, F1, F2	6 QTLs	–	Monteros et al. (2006)
KS 4895 × Jackson	RIL (92)	Canopy wilting	Satt177, Satt362	SSR	A2, B2, D2, F	4 QTLs	47	Charlson et al. (2009)
Kefeng1 × Nannong1138-2	RIL (184)	Seed yield per plant, drought susceptibility index	–	–	C2, H	10 QTLs	–	Du et al. (2009a)
Kefeng1 × Nannong1138-2	RIL (184)	Leaf water and seed yield	–	–	C2, D1b, H and A2	17 QTLs	–	Du et al. (2009b)

(continued)

Table 1.3 (continued)

Parents involved	Population/Size	Traits related to drought tolerance	Linked marker(s)	Type of marker	LG/Ch No	QTLs/genes	PVE(%)	References
PI 416,937 × “Benning”	RIL (128)	Root traits	Satt383, Satt339, Sct_191, Satt429 Sat_299	SSR	1, 3, 4, 8, 20	5 QTLs	51	Abdel-Haleem et al. (2011)
Hongfeng 11 × Clark	BC	RWC, water holding capacity, root traits canopy wilting	Sat_136, Satt167, Satt398 Satt694, GMSL514	SSR	A1, A2, B1, B2, D1a, C2, E, F, K, G, I, L M, N, O	40 QTLs	–	Li et al. (2011)
Hongfeng 11 × Clark	BC (46)	Germination rate and Seedling stage drought	Satt449, Satt499, Satt440, Sat_180	SSR	A1, K I, H	31 QTLs	–	Qiu et al. (2011)
PI 416,937 × Benning	RIL (150)	Canopy wilting	Satt 302	SSR	2, 4, 5, 12, 14, 17,19	7 QTLs	75	Abdel-Haleem et al. (2012)
Hongfeng 11 × Harosoy	BIL	–	Satt253, Satt513, Satt693, Satt240, Satt323, and Satt255	SSR	–	18 QTLs	–	Zhang et al. (2012)
SNWS 0048 × Jinda73	BIL	Physiological trait	–	–	D2, G, M, N	9 QTLs	–	Yang et al. (2014)
Jingdou23 × ZDD2315	RIL (447)	Root and shoot traits o seedlings	Satt333, Satt327, Satt519, Satt597	SSR	–	24 QTLs	7.05–38.91	Liang et al. (2014)

(continued)

Table 1.3 (continued)

Parents involved	Population/Size	Traits related to drought tolerance	Linked marker(s)	Type of marker	LG/Ch No	QTLs/genes	PVE(%)	References
93,705 KS4895 × Jackson, 08,705 KS4895 × Jackson, KS4895 × PI 424,140, A5959 × PI 416,937, Benning × PI 416,937	–	Canopy-wilting trait	–	–	2, 5, 11, 14, 17, 19	13 QTLs	–	Hwang et al. (2015)
Dunbar × PI 326582A	BIL (251)	Root and shoot traits	Satt315, Satt253, Satt142	SSR	8, 12	4 QTLs	7.7–20.8	Manavalan et al. (2015)
CPI 26,671 × G 2120; Valder × G 2120	RIL	Epidermal conductance, relative water content, plant survival after stress	soPt-856602	DArT	6	106 QTLs; 34 QTLs	–	Vu et al. (2015)
PK 1180 × UPSL 298	F_2/BSA	Seedling survivability	Satt277	SSR	6	One gene	–	Sreenivasa et al. (2020)
PI 416,997 × PI 567201D	RIL (196)	Carbon isotope discrimination	–	SNP	6, 7, 10, 11, 15, 17, 20	16 QTLs	2.5–29.9	Bazzer et al. (2020)
Water logging tolerance								
Archer × Minsoy and Archer × Minsoy	RILs (122), RILs (86)	Flooding tolerance in terms of yield and plant height	Sat_064	SSR	18	1 QTL	–	VanToai et al. (2001)

(continued)

Table 1.3 (continued)

Parents involved	Population/Size	Traits related to drought tolerance	Linked marker(s)	Type of marker	LG/Ch No	QTLs/genes	PVE(%)	References
A5403 × Archer and P9641 × Archer	RILs (103), RILs (67)	Water logging injury score	Satt269, Satt385	SSR	3, 5, 13	3 QTLs	10–16	Cornelious et al. (2005)
Misuzudaizu × Moshidou Gong 503	RILs (60)	Flooding tolerance index based on seed yield	Satt100, Sat_238, A520	SSR, RFLP, AFLP	2, 6, 7, 10, 11, 14, 19	7 QTLs	6.8–49.2	Githiri et al. (2006)
Kefeng No. 1 × Nannong	RILs (184)	Flooding toleranc in terms of plant survival rate	Satt531, Satt648, Satt038	SSR	1, 5, 18	3 QTLs	4.4–7.6	Wang et al. (2008b)
Peking × –	–	Flooding tolerance	Sat_175, Satt187, Satt338, Sat_279	SSR	2, 4, 8, 12	4 QTLs	–	Sayama et al. (2009)
Peking × Tamahomare	RILs (96)	Flooding tolerance in terms of Alcohol dehydrogenase content	Satt184, Sat_134, Sat_309	SSR	1, 8, 9, 13, 19	5 QTLs	14–35	Rizal and Karki (2011)
PI 408105A × S99-2281	RILs (188)	Flooding tolerance in terms of plant injury and yield index	BARC-016279–02, 316, BARC-024569–04, 982	SSR	11, 13	2 QTLs	7.7–19.7	Nguyen et al. (2012)
Tachinagaha × –	–	Flooding tolerance in terms of Root traits	Satt342, Satt469, Sat_177, Sat_342	SSR	4, 9, 11, 12, 13, 14	17 QTLs	1–23	Nguyen et al. (2017)

(continued)

Table 1.3 (continued)

Parents involved	Population/Size	Traits related to drought tolerance	Linked marker(s)	Type of marker	LG/Ch No	QTLs/genes	PVE(%)	References
S99-2281 × PI 561,271	RILs (182)	Flooding injury score	Gm03_3087237_A/G, Gm10_43840376_T/C	SNP	3, 10	2 QTLs	8.5–33.1	Ye et al. (2018)
5002 T × 91,210–350 and RA-452 × Osage	RILs (111), RILs (79)	Flooding damage score, Plant survival and chlorophyll content	Gm11_27896148_A_G, Gm13_28041039_G_A	SNP	1, 3, 6, 7, 11, 13, 14, 17, 19, 20	29 QTLs	9–29	Hummer (2018)
Linhe × M8206 and Zhengyang × M8206	RILs (104), RILs (126)	Seed flooding tolerance in terms of seedling length	LDB_1_46012724, LDB_13_29032373_29059156, LDB_17_8879391_9078832	SNPLDB/SNP	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 19, 20	33 QTLs	0.5–4	Ali et al. (2020)
Paldalkong × NTS1116	RILs	Foliar damage at V_1/V_2 stage	–	SNP	10, 12, 13 and other 6 chr	20 QTLs	5.8 – 33.3%	Dhungana et al. (2020)

1.8.3 QTL Mapping for Flooding Tolerance

Several studies have focused on understanding the genetic and molecular mechanisms of flooding tolerance in soybean identifying underlying major QTLs (http://www.soybase.org). VanToai et al. (2001) identified one QTL linked to molecular marker Sat_064 located on chromosome 18 associated with flooding tolerance. However, Reyna et al. (2003) could not find this QTL (Sat_064) associated to water logging tolerance in near-isogenic line (NIL) populations due to different genetic background or location/soil types of studies. Cornelious et al. (2005) reported five QTLs associated with flooding tolerance. The marker Satt485 on chromosome 3, marker Satt599 on chromosome 5, and three markers Satt160, Satt269, and Satt252 on chromosomes 13 were identified to be linked with the QTL. They are associated with flooding tolerance in two RIL populations (Table 1.3). Githiri et al. (2006) identified seven QTLs associated with yield under flooding stress resulting in a proposed QTL near Satt100. Wang et al. (2008b) mapped three QTLs, Satt531-A941V (chromosome 1), Satt648-K418_2V (chromosome 5), and Satt038-Satt275 (chromosome 18) associated with soybean flooding tolerance. Sayama et al. (2009) detected four putative QTLs viz. Sft1, Sft2, Sft3, and Sft4 associated with flooding tolerance and were mapped on to the chromosomes 2, 4, 8, and 12, respectively. Two new QTLs associated with both flooding injury score and flooding yield index were mapped on chromosomes 11 and 13 (Nguyen et al. 2012). However, these QTLs were discovered using bi-parental population characterized with a restricted mapping resolution due to limited recombination events. Later several novel QTLs associated with root system architecture, water-logging tolerance and yield in soybean have been identified (Ye et al. 2018; Wu et al. 2017b; Wu et al. 2020; Sharmin et al. 2020).

1.8.4 QTL Mapping for Salt Tolerance

Dissecting the genetic mechanism of salt tolerance in various stages of crop growth critical for the breeding of salt-tolerant soybeans (Munns and Tester 2008; Deinlein et al. 2014). Genetic architecture of salt tolerance in soybean has been dissected in several studies through bi-parental mapping and genome-wide association studies. An overview of the salt tolerant QTLs identified in soybean through bi-parental mapping is given in Table 1.4. In an $F_{2:5}$ population derived from a cross of the salt-tolerant cultivar S-100 and salt-sensitive cultivar Tokyo, Lee et al. (2004) mapped a major locus on Gm03, explaining 29% and 35% of phenotypic variation in green house and field conditions, respectively. Chen et al. (2008) identified four QTLs for salt tolerance at the seedling stage on Gm03, Gm07, Gm09, and Gm18. Subsequently, several studies have confirmed the major locus on Gm03, in different genetic backgrounds using bi-parental mapping populations, including interspecific cross mapping population of *G. max* × *G. soja* (Hamwieh and Xu 2008; Hamwieh et al. 2011; Ha et al. 2013; Qi et al. 2014; Guan et al. 2014a; Zeng et al. 2017; Do et al.

Table 1.4 Overview of QTLs identified for salt tolerance in soybean

Parents	Population type	Trait[a]	Chromosome	Nearest marker/ marker interval of QTL	LOD score	Phenotypic variance (%)	Positive allele parent	References
Kefeng No.1 × Nannong 1138–2*	RIL	ST–GR, ST–GP	Gm01	Satt468, Satt268	3.7, 4.12	13.12, 22.95	–	Zhang et al. (2019)
Kefeng No.1 × Nannong 1138–2*	RIL	ST–GP, ST–IR	Gm02	Satt611, Satt266	3.21, 3.23	9.23, 7.03	–	Zhang et al. (2019)
S-100* × Tokyo	$F_{2:5}$	LSS	Gm03	Satt237–Sat_091	7.2–12.9	29–45	S-100	Lee et al. (2004)
Kefeng 1 × Nannong 1138–2*	RIL	STR	Gm03	Satt237–Satt312	–	7.8	Nannong 1138–2	Chen et al. (2008)
Jackson × JWS156-1*	F_2	STR, SPAD	Gm03	Satt237–Satt255	43.4, 33.4	68.7, 49.6	JWS156-1	Hamwieh and Xu (2008)
FT-Abyara* × C01	RIL	STR, SPAD	Gm03	Sat_091	12.1, 10	44, 38.2	FT-Abyara	Hamwieh et al. (2011)
Jindou No. 6* × 0197	RIL	STR, SPAD	Gm03	Sat_091	11.2, 8.6	47.1, 40.9	Jindou No. 6	Hamwieh et al. (2011)
PI483463* × Hutcheson	RIL	LSS	Gm03	Satt255–BARC-038333–10,036	18.8	56.5	PI 483,463	Ha et al. (2013)

(continued)

Table 1.4 (continued)

Parents	Population type	Trait[a]	Chromosome	Nearest marker/ marker interval of QTL	LOD score	Phenotypic variance (%)	Positive allele parent	References
85–140 × Tiefeng 8*	$F_{2:3}$ and RIL	STR	Gm03	QS08064–BARCSOYSSR_03_1301	–	–	Tiefeng 8	Guan et al. (2014a)
W05* × C08	RIL	Salt damage index	Gm03	40,204,091–41,182,426	4.3	54.6	W05	Qi et al. (2014)
Williams 82 × Fiskeby III*	F2	LSS, CCR, LSC, LCC	Gm03	Salt-20–Gm03_41135466	19, 11, 7.6, 25.5	48.2, 31.3, 20.6, 58.9	Williams 82, Fiskeby III, Williams 82, Fiskeby III	Do et al. (2018)
RA-452 × Osage*	RIL	LCC	Gm03	Gm03_39491355–Gm03_41605831	13	36–54	Osage	Zeng et al. (2017)
Jidou 12* × Ji NF 58	RIL	STR, SPAD	Gm03	GMABAB–Barcsoyssr_03_1421	19.8–20.1, 10.6–11	44.3–44.7, 27–27.6	Jidou 6	Shi et al. (2018)
Kefeng 1 × Nannong 1138–2*	RIL	STR	Gm07	Satt655–Satt210	–	19.7	Nannong 1138–2	Chen et al. (2008)
Kefeng No.1 × Nannong 1138–2*	RIL	ST–IR, ST–GP, ST–GR	Gm07	Satt245, Satt245, Satt590	3.01, 3.04, 3.15	10.69, 9.08, 16.5	–	Zhang et al. (2019)

(continued)

Table 1.4 (continued)

Parents	Population type	Trait[a]	Chromosome	Nearest marker/ marker interval of QTL	LOD score	Phenotypic variance (%)	Positive allele parent	References
Kefeng No.1 × Nannong 1138–2*	RIL	ST–IR, ST–GI, ST–GP, ST–GR	Gm08	Satt162, Satt162, Satt162 and Satt310, Satt162 and BE820148	3.14–17.06, 3.34–3.72, 2.84–7.63, 8.41–8.85	6.73–46.8, 7.11–8.46, 11.2–19.8, 18–19.8	–	Zhang et al. (2019)
Kefeng 1 × Nannong 1138–2*	RIL	STR	Gm09	Sat_363–S att710	–	19.2	Nannong 1138–2	Chen et al. (2008)
Kefeng No.1 × Nannong 1138–2*	RIL	ST–GP, ST–GR	Gm10	Satt331, Sat_274	4.44, 4.8	33.4, 30.6	–	Zhang et al. (2019)
Williams 82 × Fiskeby III*	F_2	LSC	Gm13	Gm13_27665585 –Gm13_28206014	4.56	11.5	Fiskeby III	Do et al. (2018)
RA-452 × Osage*	RIL	LCC	Gm13	Gm03_39491355 – Gm03_41605831	3.2	6	RA-452	Zeng et al. (2017)
RA-452 × Osage*	RIL	LCC	Gm15	Gm15_6646246–Gm15_7147226	2.4	9	Osage	Zeng et al. (2017)
Kefeng No.1 × Nannong 1138–2*	RIL	ST–GI	Gm15	Satt606	2.64	6.02	–	Zhang et al. (2019)
Kefeng No.1 × Nannong 1138–2*	RIL	ST–GI, ST–GP, ST–GR	Gm17	Satt669, Satt669 and Sat_222, Satt669	2.56, 2.85–3.04, 3.93	10.94, 6.08–16.9, 13.73	–	Zhang et al. (2019)

(continued)

Table 1.4 (continued)

Parents	Population type	Trait[a]	Chromosome	Nearest marker/ marker interval of QTL	LOD score	Phenotypic variance (%)	Positive allele parent	References
Jackson × JWS156-1*	RIL	STR	Gm17	Satt447	14.5	50.2	JWS156-1	Tuyen et al. (2010)
		SPAD			11.8	43.0		
Kefeng 1 × Nannong 1138–2*	RIL	STR	Gm18	Sat_164–Sat_358	–	17.9	Nannong 1138–2	Chen et al. (2008)
Kefeng No.1 × Nannong 1138–2*	RIL	ST–GR	Gm18	Sat_358	2.5	6.74	–	Zhang et al. (2019)

[*] Salt tolerant parent. [a] STR–Salt tolerance rating, PPS–Percentage plant survival, SPAD–Leaf chlorophyll content (SPAD value), LSS–Leaf scorching score, CCR–Chlorophyll content ratio, LSC–Leaf sodium content, LCC–Leaf chloride content, ST-IR: the ratio of the imbibition rate under salt conditions to the imbibition rate under no-salt conditions, ST-GI: the ratio of the germination index under salt conditions to the germination index under no-salt conditions, ST-GP: the ratio of the germination potential under salt conditions to the germination potential under no-salt conditions, ST-GR: the ratio of the germination rate under salt conditions to the germination rate under nosalt conditions

2018; Shi et al. 2018). Zeng et al. (2017b) identified two new QTLs for leaf chloride content on Gm13 and Gm15, using KCl and NaCl treatments. Do et al. (2018) identified a QTL for salt tolerance on Gm13, linked with leaf sodium content.

To identify salt tolerance QTLs at the germination stage, Zhang et al. (2019) used a RIL population and mapped 25 QTLs associated with four different salt tolerance indices during the soybean germination stage. Out of 25 QTLs identified for four salt tolerance indices at seedling stage, nine QTLs were located in an overlapping region on Gm08 (named *qST-8,* Zhang et al. 2019). A wild soybean (*Glycine soja*) accession JWS156-1 with high saline and alkaline salt tolerance was identified, and a significant QTL for alkaline salt tolerance was detected on Gm17 (Tuyen et al. 2010). The QTL for alkaline salt tolerance was different from the QTL for saline tolerance found on Gm03, previously in this genotype. This study demonstrated that saline and sodic stress tolerances are controlled by different genes in soybean. DNA markers associated with these QTLs can be used for marker-assisted pyramiding of tolerance genes in soybean for both saline and sodic stresses. Bi-parental linkage mapping has successfully mapped two major locus and several minor loci for salt tolerance, however bi-parental linkage mapping can detect alleles from parents only (Korte and Farlow 2013). Nevertheless, salt-tolerant loci identified by linkage mapping are highly useful for marker-assisted selection and gene cloning.

DNA markers tightly linked with the salt tolerance QTLs and the genes characterized can be used in the selection of salt-tolerant lines. The major QTLs identified on Gm03 and Gm08, are stable QTLs identified in several studies, therefore, highly useful for MAS. Marker-assisted pyramiding of the identified major and minor QTLs may provide higher salt tolerance than single QTL. Marker-assisted development of NILs for major QTL on Gm03, and their evaluation showed higher salt tolerance (Guan et al. 2014b; Do et al. 2016), and higher grain yield in saline field conditions (Do et al. 2016; Liu et al. 2016). The salinity tolerance of tolerant NILs, NIL-T, was associated with the maintenance of seed size under salt stress and could be attributed to the ability to regulate Na^+ and Cl^- in both vegetative and reproductive tissues (Liu et al. 2016). Haplotype-based markers for the identified salt-tolerant QTLs were successfully developed and utilized for new tolerant germplasm identification (Patil et al. 2016; Kumawat et al. 2020).

1.9 Map-Based Cloning of Tolerance Genes

1.9.1 Strategies: Landing and Walking

Availability of genomic clone libraries with large DNA inserts is one of the essential requirements for plant genome analysis, primarily for physical mapping, gene isolation, and gene structure and function analysis. The Bacterial Artificial chromosome (BAC) vectors have been used widely for generating genomic DNA libraries in economically important crop plants including soybean. Development of BAC libraries is considered as critical step towards physical mapping and positional cloning of important genes.

1.9.2 Libraries: BAC/YAC Libraries

Several BAC libraries have been developed from different soybean genotypes and wild species. These soybean BAC libraries have been developed with different objectives including general genomic research as well as specifically for cloning of biotic and abiotic stress tolerance loci. These libraries have provided a good resource for positional cloning of agronomical and biologically important QTL genes that the representative genotype possesses. BAC libraries have also been constructed for several wild species including *G. soja, G. syndetika, G. canescens, G. stenophita, G. cyrtoloba, G. tomentella, G. falcata*, and the polyploid, *G. dolichocarpa.* All BAC libraries are publicly available to soybean researchers. The physical map generation of soybean was initiated with the development of early genetic maps characterized by the even distribution on the whole genome of the crop. Yeast artificial chromosomes (YACs) were initially developed with a view to utilize the resource for chromosome walking and in situ hybridization (Zhuet al. 1996). BAC libraries covering the whole soybean genome were generated by early genomic researchers (Marek and Shoemaker 1997; Danesh et al. 1998; Tomkins et al. 1999; Salimath and Bhattacharyya 1999; Meksem et al. 2000). BAC libraries encompassing variety of genotypes have led to the development of early physical contigs (Marek and Shoemaker 1997). Efforts were made to develop physical map of soybean genome using BAC and BIBAC based libraries (Wu et al. 2004). A physical map of soybean cultivar Williams 82 was in place that was generated from 67,968 BAC clones from a *Bst*YI library and 40,320 clones from a *Hind*III library ([http://soybeanphysicalmap.org/]). Furthermore, SSR markers derived from BAC ends sequence (BES) were mapped and integrated into the physical map to improve its quality (Shoemaker et al. 2008). Six-dimensional BAC clones pools were employed to demonstrate the anchoring of genetic markers to the soybean BAC clones (Wu et al. 2008). On the parallel lines soybean unigene sets from NCBI were computationally anchored to Williams 82 BES resulting in anchoring of additional 305 contigs thereby complementing 1,184 anchored contigs achieved through 6-D pool screening efforts (Wu et al. 2008). Thus, the physical framework was accomplished by associating the contigs to the molecular markers which in turn was achieved by finger printing of the BAC clones through overgo hybridization, RFLP hybridization and SSR amplification (Song et al. 2004). The soybean physical map was updated and available at Soybean Breeders Toolbox (SBT) in soybase website (http://www.soybase.org) for the greater benefit of research community. Later, physical maps of soybean and related wild species were used for comparative and functional genomics studies (Innes et al. 2008; Ha et al. 2012; Ashfield et al. 2012).

1.10 Genomics-Aided Breeding for Tolerance Traits

1.10.1 Details of Genome Sequencing

Soybean genome sequencing project was accomplished by US Department of Energy-Joint Genome Initiative (DOE-JGI)-Community Sequencing Program (CSP) (Schmutz et al. 2010). Peptides from other flowering plants, TIGR legume EST data base were used and aligned with soybean genome data to obtain the gene rich regions. The resultant regions were fed in to the gene prediction algorithms to find putative genic regions. The homologous regions were integrated with EST sequences using PASA program (Haas et al. 2003). The genome sequence data and gene annotation of soybean is housed in Phytozome database (Goodstein et al. 2012) (http://www.phytozome.net/). It provides access to genes and gene families either by keyword-based search or sequence similarity-based programs like BLAST and BLAT (BLAST like Alignment Tool). The sequence analysis via shared functional domain or consensus sequence similarity enables the study on the evolutionary history of each gene family and identification of the closely linked gene families. Gbrowser in the database facilitates EST alignments, utility of VISTA tracks that helps in assessing the extent of nucleotide conservation in related plant genera. The Biomart- open-source data retrieval software allows the research community to download complete data from phytozome.

1.10.2 Application of Structural and Functional Genomics in Genomics-Assisted Breeding

New sequencing technologies have the potential to rapidly change the molecular research landscape in soybean (Lam et al. 2010; Libault et al. 2010; Li et al. 2013; Chung et al. 2014). Several research projects include genome re-sequencing, gene expression, and whole transcript profiling have provided large scale datasets for comparative and functional genomics studies (Valliyodan et al. 2016, 2019; Kim et al. 2019; Kajiya-Kanegae et al. 2021). Structural variations play important roles in driving genome evolution and gene structure variation which in turn contribute to agronomic trait variations. Liu et al. (2020) selected 26 accessions and performed de novo genome assembly for soybean accession. Through a comparative genome analysis, a total of 14,604,953 SNPs and 12,716,823 Indels, 27,531 copy number variations and 723,862 present and absent variations, were identified.

In addition to structural variations, gene expression studies are imperative constituent of any crop improvement program. Expression studies on single and global gene expression pattern analysis is an integral part of any crop improvement program. The gene expression patterns are investigated using the global expression analysis techniques like high-density expression arrays, Serial Analysis of Gene Expression and other functional genomics approaches. Usage of microarray on soybean gene expression studies were conducted for functional studies of key genes (Maguire et al. 2002; Thibaud-Nissen et al. 2003; Vodkin et al. 2004).). Functional genomics studies were also conducted to identify the role of microRNAs. MicroRNAs (miRNAs) are key regulators of gene expression and play important roles in many aspects of plant biology. Turner et al. (2012), identified number of novel miRNAs and previously unknown family members for conserved miRNAs in the recently released soybean genome sequence. They classified all known soybean miRNAs based on their phylogenetic conservation (conserved, legume- and soybean-specific miRNAs) and examined their genome organization, family characteristics and target diversity. Comparative and functional genomics have been applied extensively in soybean for identification of genes associated with key agronomic and physiological traits and for understanding the genome structure (Ma et al. 2010; Livingstone et al. 2010; Kim et al. 2010; Deshmukh et al. 2014; Ratnaparkhe et al. 2013; Valliyodan et al. 2016; Li et al. 2017; Zhou et al. 2019; Kim et al. 2019; Lin et al. 2019; Ferreira-Neto et al. 2019; Schmutz et al. 2019; Chaudhary et al. 2019; Paganon et al. 2020; Liu et al. 2020a; Valliyodan et al. 2021).

1.10.3 Transcriptomic Approaches to Dvelop Drought Tolerance

Characterization of genetic elements defining the root traits and related transcriptional responses to drought tolerance has gained greater interests in soybean (Thao et al. 2013). Initial exploration of genetic tool box for drought tolerance in soybean showed strong upregulation of around 3000 root-derived genes and metabolite coumestrol (Tripathi et al. 2016). In another study, a complex response of root tissues subjected to drought tolerance was identified along with the involvement of multiple biochemical pathways (Stolf-Moreira et al. 2010). In addition, early transcriptional responses of soybean roots to drought stress have been investigated in detail by Neto et al. (2013). Further, molecular basis of canopy wilting tolerance was studies through whole transcriptome sequences of leaf tissues of contrasting soybean genotypes (Prince et al. 2015b). Among the various differentially expressed genes, gene encoding UDP glucuronosyl transferase was specific to the drought tolerant line PI 567690. Comparison of root transcriptome profiles of genotypes DT2008 and William 82 indicated that the drought tolerant ability of DT2008 roots could be ascribed to the expression of high number of genes of root origin during early dehydration than during the prolonged dehydration. Also, differential expression of genes involved in osmo-protectant biosynthesis, transcription factors among others conferred drought tolerance (Ha et al. 2015). Root-specific transcriptome changes were observed in soybean lines subjected to drought stress. It identified several transcription factors that were differentially regulated during drought stress paving way for development of transcription factor-cis element network (Song et al. 2016b).

To gain further molecular insights about the aquaporin family proteins (AQPs), the plant specific AQPs, 23 soybean tonoplast intrinsic proteins (TIPs) genes were analyzed (Song et al. 2016b). Analysis identified 81 SNPs and many InDels in coding regions of TIP genes and their functional validation have provided key information regarding the roles of AQPs in soybean under various abiotic stresses (Song et al. 2016b). Similarly, exploration of AQPs in *Glycine soja* yielded 62 *GsAQP* genes. Comparative expression and protein–protein interaction analysis of AQPs in cultivated and wild soybean have helped in identifying *GmTIP2;1* as a novel candidate gene, conferring salt and water stress tolerance (Zhang et al. 2017). The comprehensive list of investigations exploring the drought tolerance mechanism in soybean utilizing transcriptomic approaches are presented in Table 1.5.

1.10.4 Applications of Structural and Functional Genomics

Plants have evolved an integrated strategy including signal perception and transduction, regulation of gene expression and biochemical and physiological responses adapting to drought stress. An effective and direct strategy to endure drought stress is to reduce water loss through closing stomata. The stomatal aperture is modulated by multiple factors including environmental signals, biotic/abiotic stress, CO_2 concentration, light and plant hormones. Several hormones are involved in stomatal regulation, among which the stress hormone abscisic acid (ABA) plays the main role. During the signal transduction and adaptive response, the expressional changes of a large number of drought responsive genes occur. Chen et al. (2020) identified soybean drought-tolerant genotypes and new candidate genes for breeding. Total 422 SNPs and 302 genes were correlated with drought associated traits through GWAS studies. In addition, thirteen genes were identified which were associated with the node number of main stem trait. By qRT-PCR, the expression level of Glyma.03G018000 and Glyma.03G018900 in drought-tolerant varieties was significantly increased. This study provides important drought-tolerant genotypes, traits, SNPs and potential genes, possibly useful for soybean genetic breeding.

1.10.4.1 Reverse Genetics Approaches

Recent advances in gene isolation, plant transformation, and genetic engineering are being used extensively to alter metabolic pathways in plants by tailor made modifications to single or multiple genes. Many of these modifications are directed toward increasing the nutritional value of plant-derived foods and feeds. These methodologies are based on quickly growing information based on molecular findings, understanding, and predictions of metabolic fluxes and network pathways. The application of recombinant DNA and related techniques to plants opened up the potential to improve agronomic characters, drought tolerance, heat tolerance and salt stress resistance.

Table 1.5 Differentially expressed genes related to abiotic stress tolerance in soybean

Abiotic stress	Genotype(s) investigated	Tissue	Major finding(s)	References
Drought				
Drought related trait	–	Root tips	Genes related to membrane transport, defence signaling and metabolism associated to roots	Haerizadeh et al. (2011)
Drought-late vegetative (V6) to reproductive stage (R2)	Williams 82	Leaf	Both conserved and non-conserved pathways might be involved in drought tolerance in soybean Expression of *GmNAC* and hormone-related genes were modulated during drought	Le et al. (2012)
Canopy wilting tolerance	Drought-susceptible (DS) Pana and drought-tolerant (DT) PI 567,690	Leaf	Down-regulated genes (protein binding, hydrolase activity, carbohydrate/lipid metabolism,xyloglucan endo-transglycosylases associated with cell-wall, apoplast, and chlorophyll a/b binding proteins). Upregulated genes (ion binding and transport, the oxido-reductive process), SNPs in aquaporins genes	Prince et al. (2015b)
Long term and short term Dehydration and drought	Williams 82 and DT2008	Root	Early dehydration and short-term dehydration caused expression of more number of genes in DT2008 than in model Williams 82	Ha et al. (2015)

(continued)

Table 1.5 (continued)

Abiotic stress	Genotype(s) investigated	Tissue	Major finding(s)	References
Water-deficit stress (Different intensities)	Williams 82	Root	Root–specific transcriptome changes. Genes associated with hormones, carbohydrate metabolism, cell wall synthesis. Transcription factors and Cis-elements network	Song et al. (2016)
Combined drought and heat stress	Drought-resistant cultivar ‘Heinong44’	Leaf	Genes involved in defence, photosynthesis, metabolic pathways differentially regulated. Combined stresses did not cause additive genetic response	Wang et al. (2018b)
Drought stress	Cultivar Prima 2000	Crown nodules	Expression of C1 cysteine protease (*Glyma.14G085800*) and two vacuole processing enzymes (VPEs), [*Glyma.17G230700 and Glyma.05G055700*], were highly up-regulated. C1 cysteine protease (*Glyma.14G085800*)-indicator of drought-induced premature senescence of root nodules	Cilliers et al. (2018)
Drought	Williams 82	Leaf	Increase in proline (Pro) content and decreased malondialdehyde content	Shi et al. (2018)
Drought	Williams 82	Leaf	Physiological and transcriptomic responses of soybean leaves during Reproductive stage (R2) to drought	Xu et al. (2018)

(continued)

Table 1.5 (continued)

Abiotic stress	Genotype(s) investigated	Tissue	Major finding(s)	References
Dehydration stress at V2 developmental stage	Embrapa 48’ (drought-tolerant) and ‘BR 16’ (drought-sensitive)	Leaf	Utilized both the High-Throughput Serial Analysis of Gene Expression and the RNA-Seq approaches to decipher the role of inositol and inositol phosphates and raffinose family oligosaccharides pathways in dehydration stress	Ferreira-Neto et al. (2019)
Drought	*Glycine soja* drought-tolerant (PI342618B/DTP and A214/DTL) and drought-sensitive (NN86-4/DSP and A195/DSL)	Root	547 differentially expressed genes showed consistently contrasting expression between the genotypes. Twenty-eight DEGs were found located on chromosomal region rich in drought stress associated QTLs and “Hot spot regions”	Aleem et al. (2020)
Salinity				
Alkalinity ($NaHCO_3$-50 mmol/L)	*Glycine soja*	Roots	Affymetrix® Soybean GeneChip®-based expression profiling revealed upregulation of 3307 probes and 5720 down-regulation. Series of molecular events starting from signal transduction through changes in metabolism, defence, intracellular traffic leading to modulations in protein synthesis	Ge et al. (2010)

(continued)

Table 1.5 (continued)

Abiotic stress	Genotype(s) investigated	Tissue	Major finding(s)	References
Salinity (75 mM NaCl (low stress), and 150 mM NaCl (high stress)	Lindou 10, Qihuang 34, Weidou 8	Roots	Identified key genes (Glyma06G01990, Glyma08G22730, Glyma019G05140, and Glyma06G20160) involved in salt stress tolerance in soybean	Li et al. (2020c)
Soybean under salt (120 mM NaCl), saline–alkali (70 mM NaCl and 50 mM $NaHCO_3$) and drought (2% PEG 8000)	Inbred line HJ-1	Leaves and roots	Calcium signaling and nucleic acid pathway genes are upregulated in all the three stresses indicating molecular cross-talk	Fan et al. (2013)
$NaHCO_3$ stress (90 mM) at two time points (12 and 24 h after treatment)	Wild soybean variety N24852 and Lee 68 (control)	Roots	Identified DEG related to TFs, and ion transport, KEGG pathway analysis showed upregulation of phenylpropanoid biosynthesis" and "phenylalanine metabolism" pathways in soybean in response to alkalinity	Zhang et al. (2016)
200 mM NaCl solution for 7 days	Williams 82	Leaf	Increase in proline (Pro) content and decreased malondialdehyde content	Shi et al. (2018)

RNAi Technology

In functional genomics, RNA interference (RNAi) is a propitious gene regulatory approach that plays a substantial role in crop improvement by permitting downregulation of gene expression by small molecules of interfering RNA without affecting the expression of other genes. The discovery and study of the RNA interference phenomenon, in which double- stranded RNAs (*ds*RNA) elicits degradation of a target mRNA containing homologous sequence, led to development of more effective *ds*RNA-mediated gene silencing methods. RNAi is a less complicated, quick and efficient method of silencing gene expression in a range of organism including prokaryotes and eukaryotes. The silencing of a gene is a result of degradation of RNA into short RNA fragments that binds to specific nuclease which activates ribonucleases to target homologous mRNA. Specific gene silencing has been shown to be related to two ancient processes, co-suppression in plants and quelling in fungi, and has also been associated with regulatory processes such as transposon silencing, antiviral defense mechanisms, gene regulation, and chromosomal modification (Agrawal et al. 2003). The insertion of a functional intron region in the nuclear genome as a spacer fragment additionally increases the efficiency of the gene silencing induction, due to generation of an intron spliced hairpin RNA (*ihp*RNA) (Wesley et al. 2003). In plants, biotic stress is caused by living organisms, especially, viruses, bacteria, fungi, insects, arachnids, nematodes, and weeds. These organisms account for about a 40% loss in the overall yield of six major food and cash crops. RNAi technology has opened up new prospects for crop protection against biotic stresses.

Plants in their natural field conditions constantly get exposed to various abiotic factors such as high salinity, variation in temperature, flood, drought, and heavy metals, which hinders proper growth and development in plants. These factors are also one of the major causes behind huge crop losses globally. The changing climatic conditions and rapidly expanding population demand creates an urgent need to develop more stress-tolerant cultivars. Hence, RNAi technology can be utilized to develop transgenic cultivars that can cope with different abiotic stresses. Functional genomics studies revealed that novel genetic determinants are involved in stress adaptation in plants, which can be used to attain stress tolerance.

Receptor for activated C-kinase 1 (RACK-1) is a highly conserved scaffold protein that plays a significant role in plant growth and development. Rice plants generated through transgenic method (RNAi technology- a reverse genetic approach) where RNAi mediated downregulation of RACK-1 gene carried out, has shown more tolerance to drought dress as compared to the non-transgenic rice plants (Li et al. 2009). Likewise, disruption of rice farnesyltransferase/squalene synthase (SQS) by maize squalene synthase via RNAi, resulted in enhanced drought tolerance at vegetative and reproductive stages (Manavalan et al. 2012).

Stress tolerance and development in plants are regulated by miRNA and negatively affect the expression of the post-transcriptional gene. Wang et al. (2011a) examined that miRNA are involved in the very early stage during seed germination and identified that miRNA-mediated regulation of gene expression is present in maize imbibed seed. Wang et al. (2011b), reported 32 known members of 10 miRNA families and 8 new miRNAs/new members of known miRNA families that were found to be responsive to drought stress by high-throughput sequencing of small RNAs from *Medicago truncatula*. These findings suggest the importance of miRNAs in the response of plants to abiotic stress in general and drought stress in particular.

OsTZF1 gene is a member of the CCCH-type zinc finger gene family in rice (*Oryza sativa*). Conditions like drought, high-salt stress, and hydrogen peroxide can induce the expression of OsTZF1. Expression of *OsTZF1* gene was also induced by abscisic acid, salicylic acid, and methyl jasmonate. *OsTZF1* gene overexpressed transgenic plants showed enhanced tolerance to high salt and drought stresses; whereas transgenic rice plants in OsTZF1 gene were silenced using RNAi technology has shown less tolerance. This suggests the role played by OsTZF1gene in abiotic stress tolerance (Jan et al. 2013). Dehydrin proteins play a significant role in protecting plants from osmotic damage. Various research results suggest that overexpression of dehydrin gene WZY2 provides more tolerance to plant against osmotic stress. A study conducted by Yu et al. (2019) suggests that RNAi mediated silencing of WZY2 gene in Arabidopsis thaliana makes plant intolerant to osmotic stress.

Several researchers have focused on functional genomics studies of drought responsive genes (Le et al. 2012; Barbosa et al. 2013; Hua et al. 2018; Wang et al. 2018a; Wei et al. 2019). Drought responsive genes consist of regulatory genes encoding plenty of transcription factors (TFs), effector genes encoding chaperones, enzymes and ion/water channels etc. Several groups of TFs, such as ABA-responsive element-binding (AREB), dehydration responsive element binding (DREB), MYB, bZIP, NAC, and WRKY, respond to drought stress and act in an ABA-dependent or ABA-independent manner. Transcription factors are being used to develop genetically modified plants more tolerant to abiotic stresses. DREB and AREB TFs were introduced in soybean showing improved drought tolerance, under controlled conditions. Soybean, transgenic lines containing AtDREB*1A*, showed higher survival rate after a severe water deficit and important physiological responses to water deprivation, such as higher stomatal conductance and the maintenance of photosynthesis and photosynthetic efficiency (Polizel et al. 2011; de Pavia Rolla et al. 2014). Higher survival rates of DREB plants are because of lower water use due to lower transpiration rates under well-watered conditions. In addition to physiological studies, molecular analysis revealed that drought-response genes were highly expressed in DREB1A plants subjected to severe water deficit (Polizel et al. 2011). Mizoi et al. (2012) identified *GmDREB2A*, and showed that its heterologous expression in *Arabidopsis* induced stress-inducible genes such as *RD29A, RD29B, HsfA3*, and *HSP70* and improved stress tolerance. These findings indicate that plants overexpressing *AtDREB2A* and *DREB2Alike* proteins have increased tolerance to abiotic stresses. In soybean, the overexpression of *AREB1* gene indicated drought tolerance and exhibiting no leaf damage. It showed better growth and physiological performance under water-deficit as compared to the wild type (Barbosa et al. 2013).

Other transcription factor, WRKY, plays important roles in response to various abiotic stresses (Zhou et al. 2008). Previous studies have proved that soybean

GmWRKY54 can improve stress tolerance in transgenic *Arabidopsis*. Soybean transgenic plants were generated and further investigated for biological mechanisms of GmWRKY54 in response to drought stress (Wei et al. 2019). This study demonstrated that expression of *GmWRKY54*, driven by either a constitutive promoter (p*Cm*) or a drought-induced promoter (*RD29a*), confers drought tolerance. Recently, genes as candidate biomarkers have also been identified to screen for drought-tolerant genotypes (Hua et al. 2018). Using a GeneChip Soybean Genome Array, Hua et al. (2018) identified 697 differentially expressed genes. These genes are mainly involved in the metabolic and hormone signaling pathways. Ten DEGs were validated in a sample of 20 soybean cultivars varying in the level of drought tolerance. This research provided a new set of transcriptomic data and biomarkers for early diagnosis of drought damage and molecular breeding of drought tolerance in soybean.

Major advancement has also been made in the structural and functional genomics studies for salt tolerance (Roy et al. 2014; Wang et al. 2018b; Zhang et al. 2019; Li et al. 2020a, b). Several loci for salt tolerance have been mapped in soybean and among them candidate genes for two major loci have been cloned (Guan et al. 2014b; Qi et al. 2014; Do et al. 2016; Zhang et al. 2019). A major and consistent salt tolerance locus on Gm03, was fine mapped and candidate gene was cloned and characterized as a sodium transporter (Guan et al. 2014b; Qi et al. 2014; Do et al. 2016; Patil et al. 2016). Qi et al. (2014) fine mapped and identified the gene underlying this QTL in a salt tolerant wild soybean accession W05. The candidate gene named *GmCHX1*, is a counterpart of *Glyma03g32900* in Williams 82 and homolog of the Na^+/H^+ antiporter gene family. Genomic sequence analysis of *GmCHX1* for W05 and Williams 82 revealed that Williams 82 had a ~3.8 Kb Ty1/copia retrotransposon inserted into exon 3, but not in its counterpart *Glysoja01g005509* in W05 (Qi et al. 2014). In another study, Guan et al. (2014b) resolved this QTL into a salt tolerant variety Tiefeng 8, identifying the same gene *Glyma03g32900* (named as *GmSALT3*) having similar insertion of a 3.78-kb copia retrotransposon in exon 3 of salt sensitive parent. Subsequently, Do et al. (2016) characterized this locus in salt tolerant cultivar FT-Abhayra and identified *Glyma03g32900* (named *Ncl)* as causal gene. Insertion of a ~3.8-kb Ty1/copia type retrotransposon was responsible for the loss of gene function and salt sensitivity. Association of *Glyma03g32900* functional alleles and salt tolerance was confirmed in near isogenic lines (Guan et al. 2014b; Do et al. 2016). genetic Overexpression of *Glyma03g32900* by genetic transformation in the sensitive genotype Kariyutaka showed improved salt tolerance (Do et al. 2016). Fine mapping of major locus for salt tolerance *qST-8* was conducted and a candidate gene *Glyma.08g102000* (named *GmCDF1*), belonging to the cation diffusion facilitator (CDF) family, was identified (Zhang et al. 2019). RNA interference mediated downregulation of *GmCDF1* in soybean hairy roots resulted in tolerance to salt stress (Zhang et al. 2019).

1.11 Recent Concepts and Strategies Developed

Genomic-assisted breeding, genomic selection (GS), genome sequencing, marker-assisted selection (MAS), genetic engineering approaches, and genomics tools have been used to improve soybean yield and quality. Genomic selection is a simple, reliable, and powerful approach that enables the rapid selection of superior genotypes, bringing bigger benefits to the breeders. The marker-assisted selection also has an advantage in screening difficult traits and identification of recessive alleles. Recent advancement in genomic tools and next-generation sequencing techniques makes it easier to develop new varieties with the superior trait. Genomic approaches, along with bioinformatics tools, allow a gigantic leap forward in plant breeding. Genomic designing overcomes the limitations of traditional breeding methods and accelerated the development of climate-smart soybean crops. Developing abiotic stress-tolerant soybean varieties have become convenient with the availability of a complete genomic sequence of soybean. Recently, gene editing tools such as modified meganucleases, hybrid DNA/RNA oligonucleotides, zinc finger nucleases, TAL effector nucleases and modified CRISPR/Cas9 are used for developing abiotic stress tolerance (Bao et al. 2021). Each of these tools has the ability to precisely target one specific DNA sequence within a genome and to create a double-stranded DNA break. DNA repair to such breaks sometimes leads to gene knockouts or gene replacement by homologous recombination. Genome rearrangements are also possible to engineer. Creation and use of such genome rearrangements, gene knockouts and gene replacements by the soybean researchers is gaining significant momentum (Carrijo et al. 2021).

1.11.1 Genome Editing—A Magic Bullet

Genome editing is at the dawn of its golden age. It is described as the ability to modify and manipulate DNA sequences with higher precision in living cells (Segal and Meckler 2003). The ability to remove, insert or even edit DNA sequences easily and accurately has attracted the interest of the scientific community in a wide range of biotechnology areas, such as medicine, environmental studies and even agriculture. Targetable nucleases enable scientists to target and modify theoretically any gene in any organism. In the past few years, rapid development of molecular understanding with the aid of advanced computational technology and instrumentation with multiplexing and higher precision has led to the development of sequence specific DNA nucleases has progressed rapidly and such nucleases like zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems have been used in plant species such as Arabidopsis (Zhang et al. 2010; Li et al. 2013), tobacco (Nekrasov et al. 2013; Zhang et al. 2013), rice (Li et al. 2012;

Shan et al. 2013, 2014), barley (Wendt et al. 2013), soybean (Sun et al. 2015; Curtin et al. 2011), *Brachypodium* (Shan et al. 2013) and maize (Shukla et al. 2009).

All these nucleases involved in the genome editing technology are consist of DNA binding domains together with non-specific nuclease domains that generate double- strand breaks (DSBs). The DSBs are mainly repaired by non-homologous end-joining (NHEJ) or homologous recombination (HR) pathway (Chen and Gao 2013). NHEJ simply re-joins the broken DNA ends in an error-prone fashion and often results in small deletions or insertions. In the HR pathway, DSBs are correctly repaired using a homologous donor DNA as template. So far most genome editing has utilised the NHEJ pathway to knockout genes and only a few illustrations of gene insertion by HDR have been reported (Hyun 2020). The reasons may be that the mass of tissues to which DNA is delivered are often composed of determinate cells in which HDR is not the preferred repair mechanism.

1.11.1.1 ZFNs (Zinc Finger Nucleases)

Zinc finger proteins were considered as the very first of the “genome editing” nucleases to hit the scene in the end of the twentieth century. The Zinc finges, class of protein which is found the most commonly as a DNA binding protein domain in eukaryotes. Zinc finger nuclease (ZFN) is made up of two domains: DNA binding domain with repeated zinc fingers and FokI restriction enzyme-derived nuclease domain which is considered one of the most abundant DNA binding motifs in eukaryotic genome having the ability to recognize any sequence (Bitinaite et al. 1998). It is generally comprised of ~30 amino acid modules that interact with nucleotide triplets ie codons. ZFNs have been designed in such way that that it can recognize all of the 64 possible trinucleotide combinations, and by stringing different zinc finger moieties, one can create ZFNs that specifically recognize any specific sequence of DNA triplets (Segal et al. 2003). Each ZFN typically recognizes 3–6 nucleotide triplets, binds to the nuclease functions only as dimer, are required to target any specific locus. The first half part that recognizes the sequence upstream and the later one recognize the sequence downstream of the site to be modified (Szczepek et al. 2007).

1.11.1.2 TALENs (Transcription Activator-Like Effector Nucleases)

Transcription activator-like effector nucleases (TALENs) have made a huge impact on the genomic engineering (Bedell et al. 2012). TALENs, like ZFNs contain the FokI nuclease fused to the DNA binding protein domain which can be exploited for targeted cleavage. This DNA binding domain known as Transcription activator-like effectors (TALE) derived from plant pathogenic *Xanthomonas* bacterium contains 33–35 amino acid repeat domains that recognizes a single base pair of the DNA (Joung and Sander 2013). Two hyper variable amino acids which are known as the repeat-variable di-residues (RVD) determine the TALE specificity found at positions

12 and 13. The TALE repeats use four RVD domains NN, NI, HD and NG which recognize guanine, adenine, cytosine and thymidine, respectively (Deng et al. 2012).

Although TALENs are effective tools for genome editing, there are some limitations regarding the potential target sites, such as the need for T at position 1 (Doyle et al. 2012) and the fact that some TALENs fail to cause mutations at the desired location despite of engineering nuclease and DNA binding domain. The latestly developed genome editing technology- CRISPR/Cas system seems to provide a complementary approach to ZFNs and TALENs, as it only requires the PAM (NGG) motif preceding the recognition sequence.

1.11.1.3 CRISPR/Cas System

The research into the defence mechanisms of bacteria brought CRISPR to the scientific community. First discovered in 1987, the CRISPR-Cas system is an adaptive immunity prokaryotic defence system. As a result, it has been the focus of aggressive research that provided compelling insights into its function, as well as the promise of new molecular techniques (Ishino et al. 1987). CRISPR immunity has been categorized into three stages: adaptation, expression and interference. During the adaptation stage new spacer sequences are incorporated into the CRISPR locus. During the expression stage the CRISPR locus is transcribed to generate, or mature, the CRISPR RNA (crRNA). Finally, in the interference stage the invading nucleic acid is destroyed using the processed crRNA in some form of effector complex containing Cas proteins.

The most commonly used RGN in genome editing is the Cas9 nuclease from the type II CRISPR/Cas9 system of *Streptococcus pyogenes* (Jinek et al. 2012). With this system, there are two components that enable targeted DNA cleavage: a Cas9 protein and an RNA complex consisting of a CRISPR RNA (crRNA; contains 20 nucleotides of RNA that are homologous to the target site) and a transactivating CRISRP RNA (tracrRNA). For genome engineering purposes, the system can be reduced in complexity by fusing the crRNA and tracrRNA to generate a single-guide RNA (sgRNA) (Jinek et al. 2012). Also protospacer adjacent motif (PAM) sequences (5'- NGG-3'), an essential targeting component is situated upstream of the crRNA which is recognized by the cas9. The CRISPR/Cas systems can therefore cleave 23 bps target DNA sequence.

In contrast to ZFNs and TALENs, which require recoding of proteins using large DNA segments (500–1500 bp) for each new target site, CRISPR-Cas9 can be easily altered to target any genomic sequence by changing the 20-bp protospacer of the guide RNA, which can be accomplished by subcloning this nucleotide sequence into the guide RNA plasmid backbone. The Cas9 protein component remains unchanged. This ease of use for CRISPR-Cas9 is a significant advantage over ZFNs and TALENs, especially in generating a large set of vectors to target numerous sites (Mali et al. 2013). Another potential advantage of CRISPR-Cas9 is the ability to multiplex, i.e., to use multiple guide RNAs in parallel to target multiple sites simultaneously in the same cell (Cong et al. 2013 and Mali et al. 2013). With respect to site selection, CRISPR-Cas9 compares favourably with ZFNs and TALENs. With the most flexible version of the *S. pyogenes* CRISPR-Cas system, site selection is limited to 23-bp sequences on either strand that end in an NGG motif (the PAM for *S. pyogenes* Cas9), which occurs on average once every 8 bp (Cong et al. 2013).

The targeted plant genome editing using sequence specific nucleases has a great potential for crop improvement to meet the increasing global food demands and to provide sustainable productive agriculture system. Immediately after its early use to edit the genomes of bacteria and animals (Hwang et al. 2013; Mali et al. 2013), its efficacy was validated in the model plant systems of *Arabidopsis*, rice and tobacco (Feng et al. 2013; Nekrasov et al. 2013; Xie and Yang, 2013).

1.12 Genetic Engineering for Tolerance Traits

Genetic modification of soybean utilizing various genes has resulted in the improved salt and drought tolerance traits (Table 1.6). Confirmation of drought tolerance in soybean was performed by ectopic expression of AtABF*3* Gene (Kim et al. 2018). Several genes and TFs have been ectopically expressed in other model plants to study their functional significance. For example, over-expression of soybean-derived calmodulin gene (GmCaM4) in Arabidopsis enhanced tolerance to salinity owing to upregulation of AtMYB2-regulated genes, namely P5CS1 (Δ^1- pyrroline-5-carboxylate synthetase-1) (Yoo et al. 2005). Similarly, soybean-derived S-phase kinase-associated protein 1 (SKP1) gene GmSK1 was over expressed in *Nicotiana tobacum* cv. Samsun showing improved tolerance to salinity and drought stress (Chen et al. 2018). Pitman and Läuchli (2002) suggested that genetic modification for enhanced salt tolerance is an important approach. In dry regions, irrigation of moderately salt tolerant crops with brackish water is feasible and will be helpful for increasing the crop production. Identification of orthologs and their functional analysis will provide opportunity to improve salt tolerance in soybean through genetic engineering. Based on the knowledge of monovalent cation/proton antiporter (CPA) family in *Arabidopsis*, several genes have been identified and functionally characterized for their involvement in salt tolerance in soybean. Jia et al. (2017) demonstrated that *GsCHX19.3*, a member of cation/H^+ exchanger super family from wild soybean provide tolerance to high salinity and carbonate alkaline stress. *GsCHX19.3* mediates K^+ uptake and Na^+ excretion under carbonate alkaline stress when over-expressed in Arabidopsis. Sun et al. (2019a) found that a Na^+/H^+ exchanger, *GmNHX1,* was upregulated under salt stress in soybean genotype Jidou 7. Overexpression of *GmNHX1* in *Arabidopsis*, enhances salt tolerance by maintaining K^+/Na^+ ratio in root (Sun et al. 2019b). Similarly, overexpression of transcription factor *GmNAC15*, a member of the NAC transcription factor family in soybean, enhances salt tolerance in soybean hairy roots (Ming et al. 2018).

Jia et al. (2020) characterized *GmCHX20a*, a paralog of salt tolerant gene *GmCHX1,* and found that the ectopic expression of *GmCHX20a* in soybean hairy roots and Arabidopsis led to an increase in salt sensitivity and osmotic tolerance. It

Table 1.6 Genetic engineering studies related to abiotic stress tolerance

Sl. No.	Gene and organism	Recipient genome	Features	Reference
1	*Panax ginseng* (*PgTIP1*)	Hybrid soybean line 4076 (F_5) (*G. max* N23674 × *G. soja* BB52)	Transgenic lines under salt-stress *showed* better leaf stomatal movement and water–gas exchange capacity; less absorption, transport and accumulation of Na^+, Cl^-; a lower Na^+/K^+ ratio; and enhanced enzymatic antioxidant activities. Under PEG induced stress, transgenics demonstrated greater leaf water-retention capacity and reduced cell membrane damage	An et al. (2018)
2	Soybean activated C kinase 1 (*GmRACK1*)	Zhonghuang 13	Over expression and RNAi lines of GmRACK1 were developed Silencing of GmRACK1 gene improved drought tolerance during vegetative stage	Li et al. (2018)
3	Arabidopsis thaliana:ABRE-binding factors 3 *AtABF3*	Korean cultivar 'Kwangankong'	Enhanced drought tolerance due to prevention of cell membrane damage and maintenance of chlorophyll	Kim et al. (2018)
4	Glycine max *GmFDL19 (bZIP transcription factor)*	–	Drought and salt tolerance at seedling stage. Reduced accumulation of Na^+ ion and up-regulated the expression of ABA/stress-responsive genes	Li et al. (2017)
5	Soybean *BRASSINOSTEROID-INSENSITIVE 2* (*GmBIN2*) (member of the GSK3 protein kinase family)	Transgenic Soybean hairy root	Higher root growth rate when treated with salt and mannitol	Wang et al. (2018)
6	*Glycine soja (GsWRKY20)*	Soybean	Reduced MDA, low relative permeability, and higher antioxidant enzymes	Ning et al. (2017)

(continued)

Table 1.6 (continued)

Sl. No.	Gene and organism	Recipient genome	Features	Reference
7	*Soybean NAC transcription factor GmNAC15*	Soybean hairy roots	Overexpression enhances salt tolerance in soybean hairy roots	Ming et al. (2018)
8	*Soybean Cation/H + exchanger gene GmCHX20a*	Soybean hairy roots	Overexpression leads to salt sensitivity and osmotic tolerance	Jia et al. (2020)
9	*Soybean bZIP transcription factor GmbZIP15*	C03-3 (*G. max*)	Overexpression resulted in hypersensitivity to abiotic stresses (Salt and drought)	Zhang et al. (2020)
10	*Soybean cation diffusion facilitator, GmCDF1*	Soybean hairy roots	Overexpression and RNA interference of *GmCDF1* in soybean hairy roots resulted in increased sensitivity and tolerance to salt stress, respectively	Zhang et al. (2019)
11	*Soybean NAC transcription factor GmNAC06*	Soybean hairy roots	Overexpression reduces ROS production and enhance salt tolerance	Li et al. (2020)
12	*Soybean Peroxidase Gene GsPRX9*	Soybean Hairy roots	Overexpression of the *GsPRX9* gene enhanced the salt tolerance and antioxidant response	Jin et al. (2019)

was suggested that *GmCHX20a* and *GmCHX1* together addresses both osmotic stress and ionic stress at different times of salinity stress exposure (Jia et al. 2020). Higher expression of *GmCHX20a* led to an increase in salt sensitivity and osmotic tolerance in early stage of salinity stress, whereas higher expression of *GmCHX1* protected plants via Na^+ exclusion under salt stress in later stage. Jin et al. (2019) characterized *GsPRX9*, a class III peroxidase which upregulated significantly under salt stress. Overexpression of the *GsPRX9* in soybean hairy roots resulted in higher root fresh weight, primary root length, activities of peroxidase and superoxide dismutase, and glutathione level, but had shown lower H_2O_2 content than those in control roots under salt stress. This suggests that the overexpression of the *GsPRX9* gene results in enhanced salt tolerance and activation of antioxidant response in soybean. These examples provide insight into the mechanism of salt tolerance in soybean and various genes playing important role in maintaining ion ratio and antioxidant properties in plant, which can be utilized for genetic engineering of salt tolerance in soybean. To improve salt tolerance through genetic engineering, the negative regulators of salt tolerance could be down-regulated by gene editing and positive regulators could be overexpressed through genetic transformation.

The availability of large number of salinity tolerant genotypes makes it possible to develop salt tolerant soybean cultivars. Further, genetic characterization for trait inheritance and QTL identification made it feasible to introgress single or multiple salinity stress tolerant QTLs in desirable genetic background through DNA marker-assisted backcrossing and marker assisted recurrent selection (Lee et al. 2009). Identification of progeny lines which have shown higher tolerance than tolerant parental genotypes in some of the studies indicated that when positive alleles from tolerant and susceptible parents come together, higher tolerance is achievable (Hamwieh et al. 2011; Do et al. 2018). Therefore, identification of positive alleles from both types of parents is desirable for QTL pyramiding for higher salt tolerance. It is also possible to identify different positive loci from two different tolerant genotypes to increase the threshold of stress tolerance, and in such cases QTL mapping may be performed in populations derived from tolerant × tolerant parents. Functional characterization of positive regulators of salinity stress tolerance like *GmCHX1*, *GmCHX19.3*, *GmNAC15* and *GmNHX1,* made it feasible to genetically engineer target soybean cultivars in a short period of time. However, identification of negative regulators of salinity tolerance indicates that target genetic background should be carefully characterized to overcome the negative interaction of these negative loci, when introgression or modification of positive genes and alleles is planned.

1.13 Prospectus and Limitations of Genomic Designing for Soybean

Genomic designing approaches have enabled the improvement of soybean at a faster pace than traditional approaches. Introgression of genes and QTLs become much easier with the genomics advances. Marker-based QTL mapping is a powerful method to recognize regions of the genome that co-segregate with a given trait and mapping of QTL for abiotic stress tolerance can be utilized for the elevation of tolerance against drought (Carpentieri-Pipolo et al. 2012; Zhang et al. 2012), salt (Hamwieh et al. 2011; Ha et al. 2013; Tuyenet al. 2013), flood (Guzman et al.2007; Li et al. 2008b), and heavy metal stress (Sharma et al. 2011) in soybean. QTL mapping is more efficient compared to traditional mapping approaches since it does not require large numbers of progenies and generations of segregation populations. Genome-wide association study is an excellent approach to explore the allelic diversity present in the natural accessions of soybean. Furthermore, GWAS mapping resolution is higher than QTL mapping resolution due to millions of crossing events accumulated in the germplasm in the course of evolution (Deshmukh et al. 2014). Genome-wide association study has a great advantage in the dissection of the complex genetic architecture (Korte and Farlow 2013). Genome-assisted breeding in soybean helps in selecting superior genotypes which in turn improve the quality and yield of soybean crops on a large scale.

Although genome designing approaches have many benefits and are less time-consuming, more reliable, and easier methods, it has some limitations also. For instance, the resolution of QTL mapping is not very high due to biased mapping of QTL. Also, this method is limited to map allelic diversity that tends to segregate in a biparental population (Borevitz and Nordborg 2003). From a single QTL mapping experiment, it is hard to isolate perfect candidate genes. Moreover, genes that are identified by QTL mapping experiments are limited to those that segregate in the considered cross (Brachi et al. 2010). Genome-wide association study can overcome these limitations of QTL mapping, although it has its limitations such as the risk of many false positives as a result of population structure, unpredictable power to detect QTL, and the background LD can confound the results. The main drawback of MAS is linkage drag which can be minimized by marker-assisted backcrossing (MAB) and GS limitation is high cost and low accuracy (Staub et al. 1996; Deshmukh et al. 2014). Genome editing and other genomic methods undoubtedly set a milestone that solves all new challenges in the stream of science, however, it has some major ethical issues and negative side effects. In the future, advancement in genomic designing tools and methodologies may overcome the above-mentioned limitations (Bao et al. 2021; Carrijo et al. 2021).

1.14 Bioinformatic Resources for Soybean Improvement

Bioinformatics plays an inevitable role in the modern genomics era. It is a science of collecting, storing, and developing algorithmic tools to analyze and understand complex biological data. There are several databases and bioinformatics tools available for various purposes.

1.14.1 Gene and Genome Databases

Arabidopsis was the first plant species and the third multicellular organism to be completely sequenced and published (Kaul et al. 2000). Later, with the advancement of next-generation sequencing, several plant genomes were sequenced, and most of them are available in public databases. Biological databases are stores of biological information, and are mainly of two types, primary and secondary database. In the primary database, the sequence information is stored, and the secondary database utilizes this information. The secondary database uses the genome sequence information and performs the downstream analysis like functional annotations. The most important databases where genome and gene sequences can be submitted and retrieved are NCBI, Phytozome, and Ensemble. SoyKB and SoyBase are secondary databases that are specific to soybean. Most of these databases were generated for easy retrieval of specific genomic sequences, annotated genes, and putative functions of the genes possess marker information, QTL, transcriptomic data and can perform other downstream analysis. These databases play an important role in the identification of homologous genes using the information of functionally characterized genes.

1.14.2 Comparative Genome Databases

Genome sequencing of a large number of plant species and whole-genome resequencing of different cultivars of a crop generates new scopes of comparative genomics. Several studies have been published for comprehensive gene family analysis and duplication among the plant species. These types of studies are very important for the evolutionary fingerprinting of plant species. On the other hand, whole genome resequencing helps to explore genomic variants within a species. The comparative genomic variants would help in the dissection of biochemical pathways. The variant information of around 20,000 soybean accessions is available at SoyBase (Grant et al. 2010; Brown et al. 2020; https://www.soybase.org/) and SoyKB (Joshi et al. 2017; http://soykb.org/) database generated by SoySNP50K chip (Song et al. 2013). These single nucleotide variant data can be downloaded from SoyBase and SoyKB databases using Plant Introduction (PI) ID, genomic coordinate, and SNP ID. Further, variant information can be utilized for various studies like genome-wide association study, genomic selection, and superior haplotype identification. The comparative genomic analysis also provides evolutionary information, polyploidization, copy number variation, and presence-absent variations (PAV). Ha et al. (2019) developed a database Soybean-VCF2Genomes to identify the closest accession in soybean germplasm collection.

1.14.3 Gene Expression Databases

The transcriptional data provides the information about different gene interaction in diverse biological conditions, their role in biochemical pathways, and their function. The microarray and expressed sequence tags (EST) data information was dominant over a decade. Later, the advancement of NGS techniques replaced these conventional techniques. Next-generation sequencing is based on whole tissue mRNA sequencing and generate large amounts of sequencing data related to gene expression in various environmental conditions that can play important role in predicting gene function. There are several methods for gene expression analysis such as microarrays, Gene Chips, EST, serial analysis of gene expression (SAGE), massive parallel signature sequencing (MPSS), and RNAseq (Chaudhary et al. 2015). RNA-seq data related to various environment and stress conditions are available at different public sites like NCBI (https://www.ncbi.nlm.nih.gov/sra/), EMBL-ENA (https://www.ebi.ac.uk/ena/browser/home) and DDBJ (https://www.ddbj.nig.ac.jp/index-e.html). These databases provide the RNA-seq data of sequence read archive (SRA) raw files which can be analyzed using various publicly available RNA-seq pipelines. However, some databases like BAR (http://bar.utoronto.ca/), SoyKB, and SoyBase provides the publicly available analyzed data in the form of gene expression profile in different tissues and conditions. Several studies have been performed using publicly available RNAseq data and identified various key genes related to specific conditions (Machado et al. 2019). The biotic and abiotic stress related RNAseq data is also available in future, meta-transcriptomics analysis would result in the understanding of precise gene function, gene-environment interaction, and complex biological pathways.

1.14.4 Protein or Metabolome Databases

Proteins are the most important biomolecules as they directly control biological pathways and act as a functional unit. There are several hundred different proteins present in soybean seed but the major is glycinin (11S legumin type) and conglycinin (7S vicilin type), both comprise 65–80% of total protein content and 25–35% of seed content (Hammond et al. 2003). Soybean also has anti-nutrient content like kunitz trypsin inhibitors, lectin, P34 allergen, urease, and some other transporter protein, oil storage protein oleosins, sucrose binding and many others. Many studies have been conducted in soybean and different crops for the identification of protein expression in different tissue at various time intervals under stress conditions. The different techniques like 2D gel electrophoresis, HPLC, UPLC, LCMS, and GCMS have been used for the identification of proteins/metabolome in different environmental conditions. Several metabolites are available in Kyoto Encyclopedia of Genes and Genomes (KEGG: https://www.genome.jp/kegg/), Arabidopsis acyl-lipid metabolism (http://aralip.plantbiology.msu.edu/pathways/pathways), BRENDA (https://www.brenda-enzymes.org/index.php), MassBank (http://www.massbank.jp/). Medicine plant (http://medicinalplantgenomics.msu.edu/), MetabolomeXchange (http://www.metabolomexchange.org/site/), Plant Metabolic Network (PMN: https://plantcyc.org/), Plant/Eukaryotic and Microbial Systems Resource (PMR: http://metnetweb.gdcb.iastate.edu/PMR/), PRIMe (http://prime.psc.riken.jp/?action=metabolites_index), MetaboLights (https://www.ebi.ac.uk/metabolights/index). SoyMetDB is a metabolomic database for soybean and provide a one-stop web resource for integrating, mining and visualizing soybean metabolomic data, including identification and expression of various metabolites across different experiments and time courses (Joshi et al. 2017). These databases give the idea about metabolite biochemical and physiological properties.

1.14.5 Integration of Data from Multiple Sources

The advancement of different modern techniques in genomics, proteomics, ionomics, metabolomics, and phenomics develops a large amount of data that can be integrated to find precise identification of the target. There are several studies that successfully identified target by integrating two or more techniques. The genome-wide association studies (GWAS) along with transcriptomics data have been successfully explored for the identification of candidate genes governing particular traits. A computation approach, "camoco" has been developed which is the integration of GWAS and gene co-expression network (Schaefer et al. 2018). The integrated use of GWAS and RNAseq data identified 7 promising candidate genes for drought tolerance in maize, from the 62 loci identified in GWAS (Guo et al. 2020). Similar studies are also available in *Brassica* for yield (Lu et al. 2017) and in linseed for seed fatty acid metabolism (Xie et al. 2019). In recent study, integration of GWAS, digital phenotyping and transcriptomics was done for the identification of drought resistance genes in cotton (Li et al. 2020). Further, the integration of WGRS, transcriptome, and metabolite at different seed development stages have been utilised for the dissection of seed component related traits (Chaudhary et al. 2015). SoyBase provides the data of genetics, genomics, and USDA germplasm information. The loci information of nearly 100 traits for QTLs mapping and GWAS studies are available on SoyBase (Grant et al. 2010). The SoyKB is a web-based database that provides data of genomics, transcriptomics, metabolomics, and molecular breeding (Joshi et al. 2017). A recently developed SoyTD integrated database (http://artemis.cyverse.org/soykb_dev/SoyTD/) of WGRS and transcriptomics gives the information of natural variations and expression of soybean transporter genes (Deshmukh et al. 2020). Lai et al. (2020) developed a comprehensive framework consisting of of bioinformatics big data mining, meta-analysis, and a gene prioritization algorithm. A total of 36,705 test genes set collected from multidimensional data platforms were analysed and candidate genes for flooding tolerance were identified. In the future, integration of more databases would help to accurately understand the complex biochemical pathways and identification of candidate genes for a specifictrait.

1.15 Future Perspectives

In just the past few years we have witnessed tremendous progress in soybean comparative and functional genomics and an explosive expansion of new resources. We have seen large scale whole genome sequencing, development of high-density genetic maps using high through put approaches, construction of physical and transcript maps, development of high-density cDNA and oligo arrays, and advancement in functional genomics studies. These resources and the research outcome have shed much light on the structure, organization and evolution of the soybean genome and key genes associated with biotic, abiotic stresses and other traits. With the availability of the whole-genome sequence of the soybean genome, emerging functional genomic data and large-scale re-sequencing data, genome-wide comparisons are being achieved. These approaches will allow researchers to decipher the evolutionary history and genomic complexity of soybean. We will be able to further explore genomic approaches to the elucidation of key genes or functional components that control complex agronomical and physiological traits.

References

Abdurakhmonov IY, Abdukarimov A (2008) Application of association mapping to understanding the genetic diversity of plant germplasm resources. Intl J Plant Genom 2008: 574927

Abdel-Haleem H, Lee GJ, Boerma RH (2011) Identification of QTL for increased fibrous roots in soybean. Theor Appl Genet 122(5):935–946

Abdel-Haleem H, Carter TE Jr, Purcell LC, King CA, Ries LL, Chen P, Schapaugh W Jr, Sinclair TR, Boerma HR (2012) Mapping of quantitative trait loci for canopy-wilting trait in soybean (*Glycine max* L. Merr). Theor Appl Genet 125(5): 837–846

Abel GH, Mackenzie AJ (1964) Salt tolerance of soybean varieties (*Glycine max* L. Merrill) during germination and later growth. Crop Sci 14:157–161

Agrawal N, Dasaradhi PVN, Mohmmed A, Malhotra P et al (2003) RNA interference: biology, mechanism, and applications. Microbiol Mol Biol Rev 67(4):657–685

Ahmed F, Rafii MY, Ismail MR, Juraimi AS, Rahim HA, Asfaliza R, Latif MA (2013) Waterlogging tolerance of crops: breeding, mechanism of tolerance, molecular approaches, and future prospects. Biomed Res Int 2013:1–10

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132:1131–1139

Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan PB (1995) Integration of simple sequence repeat (SSR) DNA markers into a soybean linkage map. Crop Sci 35:1439–1445

Aleem M, Raza MM, Haider MS, Atif RM, Ali Z, Bhat JA, Zhao T (2020) Comprehensive RNA-seq analysis revealed molecular pathways and genes associated with drought tolerance in Wild Soybean (*Glycine soja* Sieb. & Zucc.) Physiol Planta https://doi.org/10.1111/ppl.13219

Ali MJ, Xing G, He J, Zhao T, Gai J (2020) Detecting the QTL-allele system controlling seed-flooding tolerance in a nested association mapping population of soybean. Crop J 8(5):781–792

An J, Cheng C, Hu Z, Chen H, Cai W, Yu B (2018) The Panax ginseng PgTIP1 gene confers enhanced salt and drought tolerance to transgenic soybean plants by maintaining homeostasis of water, salt ions and ROS. Environ Exp Bot 155:45–55

Anonymous (2015) Annual Report 2014–2015. Directorate of Soybean Research, Indore

Apuya NR, Frazier BL, Keim P, Roth EJ, Lark KG (1988) Restriction fragment length polymorphisms as genetic markers in soybean, *Glycine max* (L.) Merrill. Theor Appl Genet 75:889–901

Ara R, Mannan MA, Khaliq QA, Miah MU (2015) Waterlogging tolerance of soybean. Bangladesh Agrono J 18(2):105–109

Ashfield T, Egan AN, Pfeil BE, Chen NW, Podicheti R, Ratnaparkhe MB, Ameline Torregrosa C, Denny R, Cannon S, Doyle JJ et al (2012) Evolution of a complex disease resistance gene cluster in diploid phaseolus and tetraploid glycine. Plant Physiol 159:336–354

Bailey-Serres J, Voesenek LACJ (2008) Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biol 59:313–339

Bailey-Serres J, Lee SC, Brinton E (2012) Waterproofing crops: effective flooding survival strategies. Plant Physiol 160:1698–1709

Bao A, Zhang C, Huang Y, Chen H, Zhou X, Cao D (2021) Genome editing technology and application in soybean improvement. Oil Crop Sci 5(1):31–40

Barbosa EGG, Leita JP, Marin SRR et al (2013) Overexpression of the ABA-dependent AREB1 transcription factor from *Arabidopsis thaliana* improves soybean tolerance to water deficit. Plant Mol Biol Rep 31:719–730

Bazzer SK, Kaler AS, Ray JD, Smith JR, Fritschi FB, Purcell LC (2020) Identification of quantitative trait loci for carbon isotope ratio (δ 13 C) in a recombinant inbred population of soybean. Theor Appl Genet 15:1–5

Beche E, Gillman JD, Song Q et al (2020) Nested association mapping of important agronomic traits in three interspecific soybean populations. Theor Appl Genet 133:1039–1054

Bedell VM, Wang Y, Campbell JM, Poshusta TL, Starker CG et al (2012) In vivo genome editing using a high-efficiency TALEN system. Nature 491(7422):114–118

Bhatia VS, Jumrani K, Pandey GP (2014) Evaluation of the usefulness of senescing agent potassium iodide as a screening tool for tolerance to terminal drought in soybean. Plant Knowl J 3(1):23–30

Bhatia VS, Jumrani K (2016) A maximin-minimax approach for classifying soybean genotypes for drought tolerance based on yield potential and loss. Plant Breed 136:691–700

Bhatnagar S, King CA, Purcell L, Ray JD (6–10 Nov 2005) Identification and mapping of quantitative trait loci associated with crop responses to water-deficit stress in soybean [*Glycine max* (L.) Merr.]. The ASACSSA-SSSA international annual meeting poster abstract. Salt lake city, UT, USA

Bitinaite J, Wah DA, Aggarwal AK, Schildkraut I (1998) FokI dimerization is required for DNA cleavage. Proc Natl Acad Sci USA 95:10570–10575

Blanco FF, Folegatti MV, Gheyi HR, Fernandes PD (2007) Emergence and growth of corn and soybean under saline stress. Sci Agr 64(5):451–459

Blum A, Shpiler L, Golan G, Mayer J, Sinmena B (1991) Mass selection of wheat for grain filling without transient photosynthesis. Euphytica 54:111–116

Blum A (2011) Plant breeding for water-limited environments. Springer. ISBN 978-1-4419-7490-7. https://doi.org/10.1007/978-1-4419-7491-4

Boerma HR, Specht JE (2004) Soybeans: improvement, production, and uses, 3rd edn, Agron. Monogr. No. 16. American society of agronomy, Inc./Crop science society of America, Inc./Soil science society of America, Inc., Madison, Wisconsin

Borevitz JO, Nordborg M (2003) The impact of genomics on the study of natural variation in *Arabidopsis*. Plant Physiol 32(2):718–725

Boyer JS (1982) Plant productivity and environment. Science 218:443–448

Boyer J (1983) Environmental stress and crop yields. In: Raper C, Kramer P (eds) Crop reactions to water and temperature stress in humid, temperate climates. West view Press, Boulder, CO, pp 3–7

Brachi B, Faure N, Horton M, Flahauw E, Vazquez A, Nordborg M, et al (2010) Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. PLoS Genet 6: e1000940

Broue P, Douglass J, Grace JP, Marshall DR (1982) Interspecific hybridization of soybean and perennial glycine species indigenous to Australia via embryo culture. Euphytica 31:715–724

Brown-Guedira GL, Thompson JA, Nelson RL, Warburton ML (2000) Evaluation of genetic diversity of soybean introductions and north American ancestors using RAPD and SSR markers. Crop Sci 40:815–823

Brown AV, Conners SI, Huang W, Wilkey AP, Grant D, Weeks NT, Cannon SB, Graham MA, Nelson RT (2021) A new decade and new data at SoyBase, the USDA-ARS soybean genetics and genomics database. Nucleic Acids Res 8:49

Bustingorri C, Lavado RS (2011) Soybean growth under stable versus peak salinity. Sci Agri 68:102–108

Carpentieri-Pipolo V, Pipolo AE, Abdel-Haleem H et al (2012) Identification of QTLs associated with limited leaf hydraulic conductance in soybean. Euphytica 186:679–686

Carrijo J, Illa-Berenguer E, LaFayette P et al (2021) Two efficient CRISPR/Cas9 systems for gene editing in soybean. Transgenic Res 3:239–249

Carter TE Jr, Nelson, R L, Sneller CH, and Cui Z (2004) Genetic diversity in soybean In: Boerma HR, Specht JE (eds) Soybeans: improvement, production, and uses, 3rd edn, Agron Monogra vol 16. American Society of Agronomy, Inc/Soil Science Society of America, Inc, Madison, Wisconsin, pp 303–416

Carthew RW, Sontheimer EJ (2009) Origins and mechanisms of miRNAs and siRNAs. Cell 136(4):642–655

Chaisan T, Van K, Kim MY, Kim KD, Choi BS, Lee SH (2010) *In silico* single nucleotide polymorphism discovery and application to marker-assisted selection in soybean. Mol Breed 29:221–233

Chandra S, Satpute GK, Nagar S, Singh M, Kumawat G, Rajesh V et al (2020) Reproductive stage water-logging tolerance: a critical assessment of traits in Soybean. Soybean Research (In Press)

Chaudhary J, Patil GB, Sonah H, Deshmukh RK, Vuong TD, Valliyodan B, Nguyen HT (2015) Expanding omics resources for improvement of soybean seed composition traits. Front Plant Sci 6:1021

Chaudhary J, Shivaraj S, Khatri P, Ye H, Zhou L, Klepadlo M, Dhakate P, Kumawat G, Patil G, Sonah H, Ratnaparkhe MB et al (2019) Approaches, applicability, and challenges for development of climate-smart soybean Genomic designing of climate-smart oilseed crops. Springer, Berlin, pp 1–74

Charlson DV, Bhatnagar S, King CA, Ray JD, Sneller CH, Carter TE Jr, Purcell L (2009) Polygenic inheritance of canopy wilting in soybean [*Glycine max* (L.) Merr.]. Theor Appl Genet 119:587–594

Chebrolu KK, Fritschi FB, Ye S, Krishnan HB, Smith JR, Gillman JD (2016) Impact of heat stress during seed development on soybean seed metabolome. Metabolomics 12:28

Chen Y, Chen P, de los Reyes BG (2006) Differential responses of the cultivated and wild species of soybean to dehydration stress. Crop Sci 46:2041–2046

Chen HT, Cui SY, Fu SX, Gai JY, Yu DY (2008a) Identification of quantitative trait loci associated with salt tolerance during seedling growth in soybean (*Glycine max* L). Aust J Agric Res 59(12):1086–1091

Chen L, Fang Y, Li X, Zeng K, Chen H, Zhang H, Yang H, Cao D, Hao Q, Yuan S, Zhang C (2020a) Identification of soybean drought-tolerant genotypes and loci correlated with agronomic traits contributes new candidate genes for breeding. Plant Mol Biol 102(1–2):109–122

Chen P, Sneller CH, Purcell LC, Sinclair TR, King CA, Ishibashi T (2007) Registration of soybean germplasm lines R01–416F and R01–581F for improved yield and nitrogen fixation under drought stress. J Plant Regist 1:166–167

Chen K, Gao C (2013) TALENs: customizable molecular DNA scissors for genome engineering of plants. J of Genet and Genomi 40(6):271–279

Chen Y, Chi Y, Meng Q, Wang X, Yu D (2018) *GmSK1*, an *SKP1* homologue in soybean, is involved in the tolerance to salt and drought. Plant Physiol Biochem 127:25–31

Chen HT, Cui SY, Fu SX, Gai JY, Yu DY (2008b) Identification of quantitative trait loci associated with salt tolerance during seedling growth in soybean (*Glycine max* L.). Aust J Agr Res 59:1086–1091

Chen L, Fang Y, Li X, Zeng K, Chen H, Zhang H, Yang H, Cao D, Hao Q, Yuan S, Zhang C, Guo W, Chen S, Yang Z, Shan Z, Zhang X, Qiu D, Zhan Y, Zhou XA (2020b) Identification of soybean drought-tolerant genotypes and loci correlated with agronomic traits contributes new candidate genes for breeding. Plant Mol Biol 102(1–2):109–122

Choi IY, Hyten DL, Lakshmi KM, Qijian S, Julian MC, Charles VQ, Kevin C, Lark KG, Robert SR, Mun SY, Eun YH, Seung IY, Nevin DY, Randy CS, Curtis PVT, James ES, Cregan PB (2007) A soybean transcript map: gene distribution, haplotype and SNP analysis. Genetics 176:685–696

Choi M, Yun JY, Kim JH et al (2021) The efficacy of CRISPR-mediated cytosine base editing with the RPS5a promoter in *Arabidopsis thaliana.* Sci Rep 11:8087

Chung WH, Jeong N, Kim J, Lee WK, Lee YG, Lee SH, Yoon W, Kim JH, Choi IY, Choi HK, Moon JK, Kim N, Jeong SC (2014) Population structure and domestication revealed by high-depth resequencing of Korean cultivated and wild soybean genomes. DNA Res 21(2):153–167

Cilliers M, van Wyk SG, van Heerden PDR, Kunert KJ, Vorster BJ (2018) Identification and changes of the drought-induced cysteine protease transcriptome in soybean (*Glycine max*) root nodules. Environ Exp Bot 148:59–69

Cornelious B, Chen P, Chen Y, De Leon N, Shannon JG, Wang D (2005) Identification of QTLs underlying water-logging tolerance in soybean. Mol Breeding 16(2):103–112

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339(6121):819–823

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE (1999) An integrated genetic linkage map of the soybean. Crop Sci 39:1464–1490

Cui Z, Carter TE Jr, Burton JW (2000a) Genetic base of 651 Chinese soybean cultivars released during 1923 to 1995. Crop Sci 40:1470–1481

Cui Z, Carter TE Jr, Burton JW (2000b) Genetic diversity patterns in Chinese soybean cultivars based on coefficient of parentage. Crop Sci 40:1780–1793

Cui Z, Carter TE Jr, Burton JW, Wells R (2001) Phenotypic diversity of modern Chinese and North American soybean cultivars. Crop Sci 41:1954–1967

Curtin SJ, Zhang F, Sander JD, Haun WJ, Starker C et al (2011) Targeted mutagenesis of duplicated genes in soybean with zinc-finger nucleases. Plant Physiol 156:466–473

Danesh D, Penula S, Mudge J, Denny RL, Nordstrom H, Martinez JP, Young ND (1998) A bacterial artifi cial chromosome library for soybean and identification of clones near a major cyst nematode resistance gene. Theor Appl Genet 96:196–202

de Paiva Rolla AA, Carvalho JdFC, Fuganti-Pagliarini R, Engels C, do Rio A, Marin SRR, et al (2014) Phenotyping soybean plants transformed with rd29A: AtDREB1A for drought tolerance in the greenhouse and field. Transgenic Res 23: 75–87

Deng D, Yan C, Pan X, Mahfouz M, Wang J, Zhu JK et al (2012) Structural basis for sequence-specific recognition of DNA by TAL effectors. Science 335:720–723

Deshmukh R, Sonah H, Patil G, Chen W, Prince S, Mutava R et al (2014) Integrating omic approaches for abiotic stress tolerance in soybean. Front Plant Sci 5:244

Deshmukh R, Rana N, Liu Y, Zeng S, Agarwal G, Sonah H, Varshney R, Joshi T, Patil GB, Nguyen HT (2020) Soybean transporter database (SoyTD): a comprehensive database for identification and exploration of natural variants in soybean transporter genes. Plant Physiol 171(4):756–770

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. Trends Plant Sci 19(6):371–379

Delgado MJ, Ligero F, Lluch C (1994) Effects of salt stress on growth and nitrogen–fixation by pea, faba-bean, common bean and soybean plants. Soil Biol Biochem 26:371–376

Dhanapal AP, Ray JD, Singh SK, Hoyos-Villegas V, Smith JR et al (2015) Genome-wide association study (GWAS) of carbon isotope ratio (δ^{13}C) in diverse soybean [*Glycine max* (L.) Merr.] genotypes. Theor Appl Genet 128:73–91

Dhanapal AP, Ray JD, Singh SK, Hoyos-Villegas V, Smith JR, Purcell LC, Fritschi FB (2016) Genome-wide association mapping of soybean chlorophyll traits based on canopy spectral reflectance and leaf extracts. BMC Plant Biol 16(1):174

Dhanapal AP, York LM, Hames KA, Fritschi FB (2021) Genome-wide association study of topsoil root system architecture in field-grown soybean [*Glycine max* (L.) Merr.]. Front Plant Sci. 10(11):590179

Dhungana SK, Kim HS, Kang BK, Seo JH, Kim HT, Shin SO, Park CH, Kwak DY (2020) Quantitative trait loci mapping for flooding tolerance at an early growth stage of soybean recombinant inbred line population. Plant Breed 139:626–638

Diers BW, Keim P, Fehr WR, Shoemaker RC (1992) RFLP analysis of soybean seed protein and oil content. Theor Appl Genet 83:608–612

Diers BW, Specht J, Rainey KM, Cregan P, Song Q, Ramasubramanian V, Graef G, Nelson R, Schapaugh W, Wang D, Shannon G, McHale L, Kantartzi SK, Xavier A, Mian R, Stupar RM, Michno JM, An YC, Goettel W, Ward R, Fox C, Lipka AE, Hyten D, Cary T, Beavis WD (2018) Genetic architecture of soybean yield and agronomic traits. G3: Genes Genomes Genetics 3, 8(10):3367–3375

Do TD, Chen H, Hien VT, Hamwieh A, Yamada T, Sato T, Yan Y, Cong H, Shono M, Suenaga K, Xu D (2016) Ncl synchronously regulates Na(+), K(+), and Cl(−) in soybean and greatly increases the grain yield in saline field conditions. Sci Rep 6:19147

Do TD, Vuong TD, Dunn D, Smothers S, Patil G, Yungbluth DC, Chen P, Scaboo A, Xu D, Carter TE et al (2018) Mapping and confirmation of loci for salt tolerance in a novel soybean germplasm. Fiskeby III. Theor Appl Genet 131(3):513–524

Do TD, Vuong TD, Dunn D et al (2019) Identification of new loci for salt tolerance in soybean by high-resolution genome-wide association mapping. BMC Genomics 20:318

Dong YS, Zhao LM, Liu B, Wang ZW, Jin ZQ, Sun H (2004) The genetic diversity of cultivated soybean grown in China. Theor Appl Genet 108:931–936

Donovan S, Mao Y, Orr D J, Carmo-Silva E, McCormick AJ (2020) CRISPR-Cas9-mediated mutagenesis of the rubisco small subunit family in nicotiana tabacum. Front Genome Editing 2. https://doi.org/10.3389/fgeed.2020.605614

Doyle EL, Booher NJ, Standage DS, Voytas DF, Brendel VP, Vandyk JK, Bogdanove AJ (2012) TAL effector-nucleotide targeter (TALE-NT) 2.0: tools for TAL effector design and target prediction. Nucleic acids research 40 (Web Server issue), W117–W122

Doyle JJ, Doyle JL, Harbison C (2003) Chloroplast-expressed glutamine synthetase in *Glycine* and related Leguminosae: phylogeny, gene duplication, and ancient polyploidy. Syst Bot 28:567–577

Du W, Wang M, Fu S, Yu D (2009a) Mapping QTLs for seed yield and drought susceptibility index in soybean (*Glycine max*) across different environments. J Genet Genom 36:721–731

Du W, Yu D, Fu S (2009b) Detection of quantitative trait loci for yield and drought tolerance traits in soybean using a recombinant inbred line population. J Integr Plant Biol 51:868–878

Elsheikh EAE, Wood M (1995) Nodulation and N-2 fixation by soybean inoculated with salt-tolerant Rhizobia or salt-sensitive *Bradyrhizobia* in saline soil. Soil Biol Biochem 27:657–661

Fan XD, Wang JQ, Yang N, Dong YY, Liu L, Wang FW, Wang N, Chen H, Liu WC, Sun YP, Wu JY (2013) Gene expression profiling of soybean leaves and roots under salt, saline–alkali and drought stress by high-throughput Illumina sequencing. Gene 512(2):392–402

Feng Z, Zhang B, Ding W, Liu X, Yang DL, Wei P, Cao F, Zhu S, Zhang F, Mao Y, Zhu JK (2013) Efficient genome editing in plants using a CRISPR/Cas system. Cell Res 10:1229–1232

Ferreira-Neto JRC, da Silva MD, Benko-Iseppon AM, Pandolfi V, Binneck E, Nepomuceno, AL, Abdelnoor RV and Kido EA (2019) Inositol phosphates and Raffinose family oligosaccharides pathways: Structural genomics and transcriptomics in soybean under root dehydration. Plant Gene 20:100202

Ferreira AR, Foutz KR, Keim P (2000) Soybean genetic map of RAPD markers assigned to an existing scaffold RFLP map. J Hered 91:392–396

Fried HG, Narayanan S, Fallen B (2019) Evaluation of soybean [*Glycine max* (L.) Merr.] genotypes for yield, water use efficiency, and root traits. PLoS One 14(2): e0212700

Githiri S, Watanabe S, Harada K, Takahashi R (2006) QTL analysis of flooding tolerance in soybean at an early vegetative growth stage. Plant Breed 125:613–618

Grant D, Nelson RT, Cannon SB, Shoemaker RC (2010) SoyBase, the USDA-ARS soybean genetics and genomics database. Nucleic Acids Res 38:D843–D846

Ge Y, Li Y, Zhu YM et al (2010) Global transcriptome profiling of wild soybean (*Glycine soja*) roots under $NaHCO_3$ treatment. BMC Plant Biol 10:153

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res 40(D1):D1178–D1186

Guan R, Chen J, Jiang J, Qiu L (2014a) Mapping and validation of a dominant salt tolerance gene in the cultivated soybean (*Glycine max*) variety Tiefeng 8. Crop J 2:358–365

Guan RX, Qu Y, Guo Y, Yu LL, Liu Y, Jiang JH, Chen JG, Ren YL, Liu GY, Tian L, Jin LG, Liu ZX, Hong HL, Chang RZ, Gilliham M, Qiu LJ (2014b) Salinity tolerance in soybean is modulated by natural variation in GmSALT3. Plant J 80:937–950

Guo J, Li C, Zhang X, Li Y, Zhang D, Shi Y, Song Y, Li Y, Yang D, Wang T (2020) Transcriptome and GWAS analyses reveal candidate gene for seminal root length of maize seedlings under drought stress. Plant Sci 292:110380

Guzman PS, Diers B, Neece D, St Martin S, Leroy A, Grau C et al (2007) QTL associated with yield in three backcross-derived populations of soybean. Crop Sci 47:111–122

Ha B, Vuong TD, Velusamy V et al (2013) Genetic mapping of quantitative trait loci conditioning salt tolerance in wild soybean (*Glycine soja*) PI 483463. Euphytica 193:79–88

Ha CV, Watanabe Y, Tran UT, Le DT, Tanaka M, Nguyen KH, Seki M, Nguyen DV, Tran LS (2015) Comparative analysis of root transcriptomes from two contrasting drought-responsive williams 82 and DT2008 soybean cultivars under normal and dehydration conditions. Front Plant Sci 6:551

Ha J, Abernathy B, Nelson W, Grant D, Wu X, Nguyen HT, Stacey G, Yu Y, Wing RA, Shoemaker RC, Jackson SA (2012) Integration of the draft sequence and physical map as a framework for genomic research in soybean (*Glycine max* (L) Merr) and wild soybean (*Glycine soja* Sieb and Zucc). G3: Genes Genomes Genetics 2(3):321–329

Ha J, Jeon HH, Woo DU, Lee Y, Park H, Lee J, Kang YJ (2019) Soybean-VCF2Genomes: a database to identify the closest accession in soybean germplasm collection (2019). BMC Bioinformatics 24 (20)(Suppl 13):384

Haas BJ, Delcher AL, Mount SM, Wortman JR, Smith RK Jr, Hannick LI, Maiti R, Ronning CM, Rusch DB, Town CD et al (2003) Improving the *Arabidopsis* genome annotation using maximal transcript alignment assemblies. Nucleic Acids Res 31:5654–5666

Haerizadeh F, Singh MB, Bhalla PL (2011) Transcriptome profiling of soybean root tips. Funct Plant Biol 38(6):451–461

Hao D, Chao M, Yin Z, Yu D (2012) Genome-wide association analysis detecting significant single nucleotide polymorphisms for chlorophyll and chlorophyll fluorescence parameters in soybean (*Glycine max*) landraces. Euphytica 186:919–931

Hammond EG, Murphy PA and Johnson LA, (2003) SOY (SOYA) BEANS. Properties and Analysis 5389–5392. https://doi.org/10.1016/B0-12-227055-X/01111-1

Hamwieh A, Tuyen DD, Cong H, Benitez ER, Takahashi R, Xu DH (2011) Identification and validation of a major QTL for salt tolerance in soybean. Euphytica 179:451–459

Hamwieh A, Xu DH (2008) Conserved salt tolerance quantitative trait locus (QTL) in wild and cultivated soybeans. Breed Sci 58:355–359

Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon 20(4):509–517

Herritt M, Dhanapal AP, Purcell LC, Fritschi FB (2018) Identification of genomic loci associated with 21 chlorophyll fluorescence phenotypes by genome-wide association analysis in soybean. BMC Plant Biol 18(1):312

Henshaw TL, Gilbert RA, Scholberg JMS, Sinclair TR (2007) Soyabean (*Glycine max* L Merr) genotype response to early-season flooding: I root and nodule development. J Agron Crop Sci 193(3):177–188

Hisano H, Sato S, Isobe S, Sasamoto S, Wada T, Matsuno A, Fujishiro T, Yamada M, Nakayama S, Nakamura Y, Watanabe S, Harada K, Tabata S (2007) Characterization of the soybean genome using EST-derived microsatellite markers. DNA Res 14:271–281

Hossain MM, Liu X, Qi X, Lam HM, Zhang J (2014) Differences between soybean genotypes in physiological response to sequential soil drying and rewetting. Crop J 2(6):366–380

Hua L, Challa GS, Subramanian S et al (2018) Genome-wide identification of drought response genes in soybean seedlings and development of biomarkers for early diagnoses. Plant Mol Biol Rep 36:350–362

Huang L, Zeng A, Chen P, Wu C, Wang D, Wen Z (2018) Genome-wide association analysis of salt tolerance in soybean [*Glycine max* (L) Merr]. Plant Breed 137:714–720

Hummer WS Linkage mapping for soybean (*Glycine max*) flood tolerance (2018) MSc thesis and dissertations, University of Arkansas, 3074

Hwang S, King CA, Ray JD, Cregan PB, Chen P, Carter TE, Li Z, Abdel-Haleem H, Matson KW, Schapaugh W (2015) Confirmation of delayed canopy wilting QTLs from multiple soybean mapping populations. Theor Appl Genet 128:2047–2065

Hwang W, Fu Y, Reyon D et al (2013) Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol 31:227–229

Hymowitz T (2004) Speciation and cytogenetics Soybeans: improvement, production, and uses, vol 16. pp 97–136

Hymowitz T, Singh RJ, Larkin RP (1990) Long-distance dispersal: the case for the allopolyploid *Glycine tabacina* (Labill) Benth and *G tomentella* Hayata in the West-Central Pacific. Micronesica 23:5–13

Hyten DL, Song Q, Zhu Y, Choi IY, Nelson RL et al (2006) Impacts of genetic bottlenecks on soybean genome diversity. Proc Natl Acad Sci USA 103:16666–16671

Hyten DL, Choi I-Y, Song Q, Specht JE, Carter TE, Shoemaker RC, Hwang EY, Matukumalli LK, Cregan PB (2010a) A high density integrated genetic linkage map of soybean and the development of a 1,536 Universal Soy Linkage Panel for QTL mapping. Crop Sci 50:960–968

Hyten DL, Cannon SB, Song Q, Weeks N, Fickus EW, Shoemaker RC, Specht JE, Farmer AD, May GD, Cregan PB (2010b) High-throughput SNP discovery through deep resequencing of a reduced representation library to anchor and orient scaffolds in the soybean whole genome sequence. BMC Genomics 11:38

Hyten DL, Song Q, Choi IY, Yoon MS, Specht JE, Matukumalli LK, Nelson RL, Shoemaker RC, Young ND, Cregan PB (2008) High-throughput genotyping with the goldengate assay in the complex genome of soybean. Theor Appl Genet 116:945–952

Hyun T (2020) CRISPR/Cas-based genome editing to improve abiotic stress tolerance in plants. Botanica Serbica 44(2):121–127

Innes RW, Ameline-Torregrosa C, Ashfield T, Cannon E, Cannon SB, Chacko B, Chen NW, Couloux A, Dalwani A, Denny R, Deshpande S, Egan AN, Glover N, Hans CS, Howell S, Ilut D, Jackson S, Lai H, Mammadov J, Del Campo SM, Metcalf M, Nguyen A, O'Bleness M, Pfeil BE, Podicheti R, Ratnaparkhe MB, Samain S, Sanders I, Segurens B, Sevignac M, Sherman-Broyles S, Thareau V, Tucker DM, Walling J, Wawrzynski A, Yi J, Doyle JJ, Geffroy V, Roe BA, Maroof MA, Young ND (2008) Differential accumulation of retroelements and diversification of NB-LRR disease resistance genes in duplicated regions following polyploidy in the ancestor of soybean. Plant Physiol 148:1740–1759

Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A (1987) Nucleotide sequence of the *iap* gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. J Bacteriol 169:5429–5433

Jackson AL, Burchard J, Schelter J, Chau BN, Cleary M, Lim L, Linsley PS (2006) Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. RNA (new York) 12(7):1179–1187

Jia B, Sun M, DuanMu H, Ding X, Liu B, Zhu Y et al (2017) *GsCHX193*, a member of cation/H^+ exchanger superfamily from wild soybean contributes to high salinity and carbonate alkaline tolerance. Sci Rep 7:9423

Jia Q, Li MW, Zheng C, Xu Y, Sun S, Li Z, Wong FL, Song J, Lin WW, Li Q, Zhu Y, Liang K, Lin W, Lam HM (2020) The soybean plasma membrane-localized cation/H^+ exchanger GmCHX20a plays a negative role under salt stress. Physiol Plant 171(4):714–727

Jin T, Sun Y, Zhao R, Shan Z, Gai J, Li Y (2019) Overexpression of peroxidase gene GsPRX9 confers salt tolerance in soybean. Intl J Mol Sci 20(15):3745

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(6096):816–821

Joshi T, Wang J, Zhang H, Chen S, Zeng S, Xu B, Xu D (2017) The evolution of soybean knowledge base (SoyKB) in plant genomics databases. Humana Press, New York, NY, pp 149–159

Joung JK, Sander JD (2013) TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol 14(1):49–55

Kajiya-Kanegae H, Nagasaki H, Kaga A, Hirano K, Ogiso-Tanaka E, Matsuoka M, Ishimori M, Ishimoto M, Hashiguchi M, Tanaka H, Akashi R, Isobe S, Iwata H (2021) Whole-genome sequence diversity and association analysis of 198 soybean accessions in mini-core collections. DNA Res 19(1):28

Kaler AS, Ray JD, Schapaugh WT, King CA, Purcell LC (2017a) Genome-wide association mapping of canopy wilting in diverse soybean genotypes. Theor Appl Genet 130:2203–2217

Kaler AS, Dhanapal AP, Ray JD, King CA, Fritsch FB, Purcell LC (2017b) Genome-wide association mapping of carbon isotope and oxygen isotope ratios in diverse soybean genotypes. Crop Sci 57:3085–3100

Kan G, Zhang W, Yang W, Ma D, Zhang D, Hao D, Hu Z, Yu D (2015) Association mapping of soybean seed germination under salt stress. Mol Gen Genom 290(6):2147–2162

Kaul S, Koo HL, Jenkins J, Rizzo M, Rooney T, Tallon LJ, Feldblyum T, Nierman W, Benito MI, Lin X, Town CD (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana.* Nature 408(6814):796–815

King CA, Purcell LC, Brye KR (2009) Differential wilting among soybean genotypes in response to water deficit. Crop Sci 49(1):290–298

Kofsky J, Zhang H, Song B-H (2018a) The untapped genetic reservoir: the past, current, and future applications of the wild soybean (*Glycine soja*). Front Plant Sci 9:949

Keim P, Shoemaker RC, Palmer RG (1989) Restriction fragment length polymorphism diversity in soybean. Theo App Gene 77:786–792

Keim P, Diers BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735–742

Keim P, Schupp JM, Travis SE, Clayton K, Zhu T, Shi L, Ferreira A, Webb DM (1997) A high-density soybean genetic map based on AFLP markers. Crop Sci 37:537–543

Keller R, Brearley CA, Trethewey RN, Muller-Rober B (1998) Reduced inositol content and altered morphology in transgenic potato plants inhibited for 1D-myo-inositol 3-phosphate synthase. Plant J 16(4):403–410

Kim MY, Lee S, Van K, Kim TH, Jeong SC, Choi IY, Kim DS, Lee YS, Park D, Ma J, Kim WY, Kim BC, Park S, Lee KA, Kim DH, Kim KH, Shin JH, Jang YE, Kim KD, Liu WX, Chaisan T, Kang YJ, Lee YH, Kim KH, Moon JK, Schmutz J, Jackson SA, Bhak J, Lee SH (2010) Whole-genome sequencing and intensive analysis of the undomesticated soybean (*Glycine soja* Sieb and Zucc) genome. Proc Natl Acad Sci USA 107(51):22032–22037

Kim HJ, Cho HS, Pak JH, Kwon T, Lee JH, Kim DH, Lee DH, Kim CG, Chung YS (2018) Confirmation of drought tolerance of ectopically expressed *AtABF3* gene in soybean. Mol Cells 41(5):413–422

Kim JY, Jeong S, Kim KH, Lim WJ, Lee HY, Jeong N, Moon JK, Kim N (2019) Dissection of soybean populations according to selection signatures based on whole-genome sequences. Gigascience 8(12):giz151

Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:29

Kumawat G, Maranna S, Gupta S, Tripathi R, Agarwal N, Singh V, Rajesh V, Chandra S, Kamble V, Nataraj V, Bharti A, Sharma MP, Jadhav PV, Ratnaparkhe MB, Satpute GK, Bhatia VS (2020) Identification of novel genetic sources for agronomic and quality traits in soybean using multi-trait allele specific genic marker assays. J Plant Biochem Biotechnol 30:160–171

Kuwano M, Ohyama A, Tanaka Y et al (2006) Molecular breeding for transgenic rice with low-phytic-acid phenotype through manipulating *myo*-inositol 3-phosphate synthase gene. Mol Breeding 18:263–272

Ladizinsky G, Newell CA, Hymowitz T (1979a) Giemsa staining of soybean chromosomes. J Hered 70:415–416

Ladizinsky G, Newell CA, Hymowitz T (1979b) Wide crosses in soybean: prospects and limitations. Euphytica 28:421–423

Lai MC, Lai ZY, Jhan LH, Lai YS, Kao CF (2021) Prioritization and evaluation of flooding tolerance genes in soybean [*Glycine max* (L.) Merr.]. Front Genet 11:612131

Lam HM, Xu X, Liu X, Chen W, Yang G, Wong FL, Li MW, He W, Qin N, Wang B, Li J, Jian M, Wang J, Shao G, Wang J, Sun SS, Zhang G (2010) Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. Nat Genet 42(12):1053–1059

Lark KG, Weisemann JM, Matthews BF, Palmer R, Chase K, Macalma T (1993) A genetic map of soybean (*Glycine max* L) using an intraspecific cross of two cultivars: 'Minsoy' and 'Noir 1.' Theor Appl Genet 86:901–906

Larson SR, Rutger JN, Young KA, Raboy V (2000) Isolation and genetic mapping of a non-lethal rice (*Oryza sativa* L.) low phytic acid 1 Mutation. Crop Sci 40(5):1397–1405

Le DT, Nishiyama R, Watanabe Y, Tanaka M, Seki M, Ham LH, et al (2012) Differential gene expression in soybean leaf tissues at late developmental stages under drought stress revealed by genome-wide transcriptome analysis. PLoS One 7(11):e49522

Lee JD, Shannon JG, Vuong TD, Nguyen HT (2009) Inheritance of salt tolerance in wild soybean (*Glycine soja* Sieb and Zucc) accession PI483463. J Hered 100:798–801

Lee GJ, Carter TE Jr, Villagarcia MR, Li Z, Zhou X, Gibbs MO, Boerma HR (2004) A major QTL conditioning salt tolerance in S-100 soybean and descendent cultivars. Theor Appl Genet 109:1610–1619

Lee S, Freewalt KR, McHale LK et al (2015) A high-resolution genetic linkage map of soybean based on 357 recombinant inbred lines genotyped with BARCSoySNP6K. Mol Breeding 35:58

Lenis JM, Ellersieck M, Blevins DG, Sleper DA, Nguyen HT, Dunn D, Lee JD, Shannon JG (2011) Differences in ion accumulation and salt tolerance among *Glycine* Accessions. J Agron Crop Sci 197:302–310

Li B, Chen L, Sun W, Wu D, Wang M, Yu Y, Chen G, Yang W, Lin Z, Zhang X, Duan L (2020a) Phenomics-based GWAS analysis reveals the genetic architecture for drought resistance in cotton. Plant Biotechnol J 18(12):2533–2544

Li CD, Jiang HW, Liu CY, Guo T, Xin WZ, Hong WX et al (2011) QTL identification of drought tolerance to soybean in selection population. Acta Agron Sin 37:603–611

Li D, Pfeiffer T, Cornelius P (2008a) Soybean QTL for yield and yield components associated with *Glycine soja* alleles. Crop Sci 48:571–581

Li DH, Li W, Li HY, Guo JJ, Chen FJ (2018) The soybean GmRACK1 gene plays a role in drought tolerance at vegetative stages. Russ J Plant Physiol 65:541–552

Li JF, Norville J, Aach J et al (2013a) Multiplex and homologous recombination–mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. Nat Biotechnol 31:688–691

Li M, Chen R, Jiang Q, Sun X, Zhang H, Hu Z GmNAC06 (2020b) A NAC domain transcription factor enhances salt stress tolerance in soybean. Plant Mol Biol 5

Li N, Li Z, Fan S, Pu Y, Gong Y, Tian R, Guo X, Ding H (2020c) Identification of potential key genes affecting soybean growth under salt stress via transcriptome study. Biol Planta 64:692–700

Li R, Jiang H, Zhang Z, Zhao Y, Xie J, Wang Q, Zheng H, Hou L, Xiong X, Xin D, Hu Z, Liu C, Wu X, Chen Q (2019) Combined linkage mapping and BSA to identify QTL and candidate genes for plant height and the number of nodes on the main stem in soybean. Int J Mol Sci 21(1):42

Li T, Liu B, Spalding M et al (2012) (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat Biotechnol 30:390–392

Li W, Han Y, Zhang D, Yang M, Teng W, Jiang Z, Qiu L, Sun G (2008b) Genetic diversity in soybean genotypes from north-eastern China and identification of candidate markers associated with maturity rating. Plant Breed 127:56–61

Li Y, Chen Q, Nan H, Li X, Lu S, Zhao X, et al (2017) Overexpression of *GmFDL19* enhances tolerance to drought and salt stresses in soybean. PLoS One 12(6):e0179554

Li YH, Li W, Zhang C et al (2010) Genetic diversity in domesticated soybean (*Glycine max*) and its wild progenitor (*Glycine soja*) for simple sequence repeat and single-nucleotide polymorphism loci. New Phytol 188(1):242–253

Li YH, Zhao SC, Ma JX, Li D, Yan L, Li J, Qi XT, Guo XS, Zhang L, He WM, Chang RZ, Liang QS, Guo Y, Ye C, Wang XB, Tao Y, Guan RX, Wang JY, Liu YL, Jin LG, Zhang XQ, Liu ZX, Zhang LJ, Chen J, Wang KJ, Nielsen R, Li RQ, Chen PY, Li WB, Reif JC, Purugganan M, Wang J, Zhang MC, Wang J, Qiu LJ (2013b) Molecular footprints of domestication and improvement in soybean revealed by whole genome re-sequencing. BMC Genomics 28(14):579

Li Z, Nelson RL (2002) RAPD marker diversity among cultivated and wild soybean accessions from four Chinese provinces. Crop Sci 42:1737–1744

Liang H, Yu Y, Yang H, Xu L, Dong W, Du H, Cui W, Zhang H (2014) Inheritance and QTL mapping of related root traits in soybean at the seedling stage. Theor App Genet 10:2127–2137

Libault M, Farmer A, Joshi T, Takahashi K, Langley RJ, Franklin LD, He J, Xu D, May G, Stacey G (2010) An integrated transcriptome atlas of the crop model *Glycine max*, and its use in comparative analyses in plants. Plant J 63(1):86–99

Lightfoot DA, Njiti VN, Gibson PT, Kassem MA, Iqbal JM, Meksem K (2005) Registration of the essex by forrest recombinant inbred line mapping population. Crop Sci 45:1678–1681

Lin Y, Li W, Zhang Y et al (2019) Identification of genes/proteins related to submergence tolerance by transcriptome and proteome analyses in soybean. Sci Rep 9:14688

Linkemer G, Board JE, Musgrave ME (1998) Waterlogging effects on growth and yield components in late-planted soybean. Crop Sci 38:1576–1584

Liu Y, Gai JY, Lü HN, Wang YJ, Chen SY (2005) Identification of drought tolerant germplasm and inheritance and QTL mapping of related root traits in soybean (*Glycine max* (L.) Merr.). Yi Chuan Xue Bao 32(8):855–863

Liu Y, Du H, Li P, Shen Y, Peng H, Liu S, Zhou GA, Zhang H, Liu Z, Shi M, Huang X, Li Y, Zhang M, Wang Z, Zhu B, Han B, Liang C, Tian Z (2020a) Pan-genome of wild and cultivated soybeans. Cell J 9(1):182

Liu D, Li M, Liu Y, Shi L (2020b) Integration of the metabolome and transcriptome reveals the resistance mechanism to low nitrogen in wild soybean seedling roots. Environ Exp Bot 175:104043

Liu Y, Yu L, Qu Y, Chen J, Liu X, Hong H, Liu Z, Chang R, Gilliham M, Qiu L et al (2016) GmSALT3, which confers improved soybean salt tolerance in the field, increases leaf cl- exclusion prior to Na+ exclusion but does not improve early vigor under salinity. Front Plant Sci 7(1485):1485

Liu, ZX, Li, HH, Wen, ZX, Fan, XH, Li, YH, Guan, RX, Guo, Y, Wang, SM, Wang, DC, Qiu, LJ (2017) Comparison of genetic diversity between Chinese and American soybean (*Glycine max* (L)) accessions revealed by high–density SNPs. Front Plant Sci 8:2014

Livingstone JM, Cheng KC, Strömvik MV (2010) Bioinformatics as a tool. In: Bilyeu K, Ratnaparkhe MB, Kole C (eds) Genetics, genomics, and breeding of soybean. CRC, New Hampshire, UK

Lu K, Peng L, Zhang C, Lu J, Yang B, Xiao Z, Liang Y, Xu X, Qu C, Zhang K et al (2017) Genome-wide association and transcriptome analyses reveal candidate genes underlying yield-determining traits in *Brassica napus*. Front Plant Sci 8:206

Lorenzen L, Boutin S, Young N, Specht JE, Shoemaker RC (1995) Soybean pedigree analysis using map-based molecular markers: i tracking RFLP markers in cultivars. Crop Sci 35:1326–1336

Ma J, Shoemaker R, Jackson S, Cannon S (2010) Comparative genomics. In: Bilyeu K, Ratnaparkhe MB, Kole C (eds) Genetics, genomics & breeding of soybean. CRC, New Hampshire, UK, pp 245–262

Machado FB, Moharana KC, Almeida-Silva F, Gazara RK, Pedrosa-Silva F, Coelho FS, Grativol C, Venancio TM (2019) Systematic analysis of 1,298 RNA-Seq samples and construction of a comprehensive soybean (*Glycine max*) expression atlas. Plant J 103(5):1894–1909

Maguire TL, Grimmond S, Forrest A, Iturbe-Ormaetxe I, Meksem K, Gresshoff P (2002) Tissue-specific gene expression in soybean (*Glycine max*) detected by cDNA microarray analysis 159:1361–1364

Mali P, Esvelt K, Church G (2013) Cas9 as a versatile tool for engineering biology. Nat Methods 10:957–963

Manavalan LP, Prince SJ, Musket TA, Chaky J, Deshmukh R, Vuong TD et al (2015) Identification of novel QTL governing root architectural traits in an interspecific soybean population. PLoS One 10(3):e0120490

Mansur LM, Orf JH, Chase K, Jarvik T, Cregan PB, Lark KG (1996) Genetic mapping of agronomic traits using recombinant inbred lines of soybean. Crop Sci 36:1327–1336

Marek LF, Shoemaker RC (1997) BAC contig development by fingerprint analysis in soybean. Genome 40:420–427

Matthews BF, Devine TE, Weisemann JM, Beard HS, Lewers KS, MacDonald MH, Park YB, Maiti R, Lin JJ, Kuo J (2001) Incorporation of sequenced cDNA and genomic markers into the soybean genetic map. Crop Sci 41:516–521

Maughan PJ, Saghai Maroof MA, Buss GR (1995) Microsatellite and amplified sequence length polymorphisms in cultivated and wild soybean. Genome 38:715–723

Meksem K, Zobrist K, Ruben E, Hyten D, Quanzhou T, Zhang HB, Lightfoot DA (2000) Two large-insert soybean genomic libraries constructed in a binary vector: applications in chromosome walking and genome wide physical mapping. Theor Appl Genet 101:747–755

Meng F, Zhao H, Zhu B, Zhang T, Yang M, Li Y, Han Y, Jiang J (2021) Genomic editing of intronic enhancers unveils their role in fine-tuning tissue-specific gene expression in *Arabidopsis thaliana*. Plant Cell 33(6):1997–2014

Mian M, Bailey M, Ashley D, Wells R, Carter T, Parrott W, Boerma H (1996) Molecular markers associated with water use efficiency and leaf ash in soybean. Crop Sci 36:1252–1257

Mian M, Ashley D, Boerma H (1998) An additional QTL for water use efficiency in soybean. Crop Sci 38:390–393

Ming LI, Zheng HU, Jiang QY et al (2018) GmNAC15 overexpression in hairy roots enhances salt tolerance in soybean. J Integr Agri 17(3):530–538

Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) AP2/ERF family transcription factor in plant abiotic stress responses. Biochim Biophys Acta 1819:86–96

Monteros MJ, Lee G, Missaoui AM, Carter TE, Boerma HR (2006) Identification and confirmation of QTL conditioning drought tolerance in Nepalese soybean. In: The 11th biennial conference on the molecular and cellular biology of the soybean, abstract PI471938. August 5–8. Lincoln, NE

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3:175–182

Morgante M, Rafalski A, Biddle P, Tngey S, Olivery AM (1994) Genetic mapping and variability of seven soybean simple sequence repeat loci. Genome 37:763–769

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nekrasov V, Staskawicz B, Weigel D, Jones JD, Kamoun S (2013) Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. Nat Biotechnol 8:691–693

Newell CA, Hymowitz T (1980) A taxonomic revision in the genus *Glycine* subgenus *Glycine* (Leguminosae). Brittonia 32:63–69

Neto JRCF, Pandolfi V, Guimaraes FCM, Benko-Iseppon AM, Romero C, de Oliveira Silva RL, Rodrigues FA, Abdelnoor RV, Nepomuceno AL, Kido EA (2013) Early transcriptional response of soybean contrasting accessions to root dehydration. PLoS ONE 8(12):83466

Nguyen VT, Vuong TD, VanToai T, Lee JD, Wu X, Mian MR, Dorrance AE, Shannon JG, Nguyen HT (2012) Mapping of quantitative trait loci associated with resistance to *Phytophthora sojae* and flooding tolerance in soybean. Crop Sci 52(6):2481–2493

Nguyen VL, Takahashi R, Githiri SM et al (2017) Mapping quantitative trait loci for root development under hypoxia conditions in soybean (*Glycine max* L Merr). Theor Appl Genet 130:743–755

Ning W, Zhai H, Yu J, Liang S, Yang X, Xing X, Huo J, Pang T, Yang Y, Bai X (2017) Overexpression of *Glycine soja* WRKY20 enhances drought tolerance and improves plant yields under drought stress in transgenic soybean. Mol Breed 37(2):19

Nunes ACS, Vianna GR, Cuneo F et al (2006) RNAi-mediated silencing of the *myo*-inositol-1-phosphate synthase gene (*GmMIPS1*) in transgenic soybean inhibited seed development and reduced phytate content. Planta 224:125–132

Oosterhuis DM, Scott HD, Hampton RE, Wullschleger SD (1990) Physiological response of two soybean [Glycine max L Merr] cultivars to short-term flooding. Environ Exp Botany 30:85–92

Pagano MC, Miransari M, Corrêa EJA, Duarte NF, Yelikbayev BK (2020) Genomic research favoring higher soybean production. Curr Genomics 21(7):481–490

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review ecotoxicold. Environ Safety 60(3):324

Pathan SM, Lee JD, Sleper DA, Fritschi FB, Sharp RE, Carter TE Jr, Nelson RL, King CA, Schapaugh WT, Ellersieck MR, Nguyen HT, Shannon JG (2014) Two soybean plant introductions display slow leaf wilting and reduced yield loss under drought. J Agron Crop Sci 200(3):231–236

Patil G, Do T, Vuong TD, Valliyodan B, Lee JD, Chaudhary J, Shannon JG, Nguyen HT (2016) Genomic-assisted haplotype analysis and the development of high-throughput SNP markers for salinity tolerance in soybean. Sci Rep 6:19199

Patterson PR, Hudak CM (1996) Drought-avoidant soybean germplasm maintains nitrogen fixation capacity under water stress. Plant Soil 186(1):39–43

Phang TH, Shao G, Lam HM (2008) Salt tolerance in soybean. J Integr Plant Biol 50:1196–1212

Pitman MG, Läuchli A (2002) Global impact of salinity and agricultural ecosystems In: Läuchli A, Luttge U (eds) Salinity: environment—plants—molecules Kluwer. Dordrecht, Netherlands, pp 3–20

Polizel AM, Medri ME, Nakashima K, Yamanaka N, Farias JRB, Oliveira MCN, Marin SRR, Abdelnoor RV, Marcelino-Gui-marães FC, Fuganti R et al (2011) Molecular, anatomical and physiological properties of a genetically modified soybean line transformed with rd29A:AtDREB1A for the improvement of drought tolerance. Genet Mol Res 10:3641–3656

Prince SJ, Song L, Qiu D, dos Santos JVM, Chai C, Joshi T, Patil G, Valliyodan B, Vuong TD, Murphy M (2015a) Genetic variants in root architecture-related genes in a *Glycine soja* accession, a potential resource to improve cultivated soybean. BMC Genomics 16:132

Prince SJ, Joshi T, Mutava RN, Syed N, Vitor MDSJ, Patil G, Song L, Wang J, Lin L, Chen W, Shannon JG (2015b) Comparative analysis of the drought-responsive transcriptome in soybean lines contrasting for canopy wilting. Plant Sci 240:65–78

Purcell LC, DeSilva M, King CA, Kim WH (1997) Biomass accumulation and allocation in soybean associated with genotypic differences in tolerance of nitrogen fixation to water deficits. Plant Soil 196(1):101–113

Qadir M, Quillerou E, Nangia V, Murtaza G, Singh M, Thomas RJ, Drechsel P, Noble AD (2014) Economics of salt-induced land degradation and restoration. Nat Resour Forum 38:282–295

Qi X, Li MW, Xie M, Liu X, Ni M, Shao G, Song C, Kay-Yuen Yim A, Tao Y, Wong FL, Isobe S, Wong CF, Wong KS, Xu C, Li C, Wang Y, Guan R, Sun F, Fan G, Xiao Z, Zhou F, Phang TH, Liu X, Tong SW, Chan TF, Yiu SM, Tabata S, Wang J, Xu X, Lam HM (2014) Identification of a novel salt tolerance gene in wild soybean by whole-genome sequencing. Nat Commun 5:4340

Qiu PC, Zhang WB, Li CD, Jiang HW, Liu CY, Fan DM, Zeng QL, Hu HG, Chen QS (2011) Genetic overlap of drought-tolerance loci between germination stage and seedling stage analyzed using introgression lines in soybean. Acta Agron Sin 37(3):477–483

Rabie RK, Kumazawa K (1988) Effect of salt stress on nitrogen nutrition and yield quality of nodulated soybeans. Soil Sci Plant Nutr 34:385–391

Rafalski A, Morgante M (2004) Corn and humans: recombination and linkage disequilibrium in two genomes of similar size. Trends Genet 20:103–111

Ratnaparkhe MB, RJ Singh, JJ Doyle (2010) *Glycine.* In: Kole C (eds) Wild crop relatives. Springer, Genomic and Breeding Resources pp 83–116

Ratnaparkhe MB, Ramesh SV, Giriraj K, Husain SM, Gupta S (2013) In: Gupta (ed) Advances in soybean genomics. Springer, Legumes in the Omic Era pp 41–72

Ratnaparkhe MB, Marmat N, Kumawat G, Shivakumar M, Kamble VG, Nataraj V, Ramesh SV, Deshmukh MP, Singh AK, Sonah H, Deshmukh RK, Prasad M, Chand S, Gupta S (2020) Whole genome re-sequencing of soybean accession EC241780 providing genomic landscape of candidate genes involved in rust resistance. Curr Genomics 21(7):504–511

Ray JD, Dhanapal AP, Singh SK, Hoyos-Villegas V, Smith JR, Purcell LC, King CA, Boykin D, Cregan PB, Song Q, Fritschi FB (2015) Genome-wide association study of ureide concentration in diverse maturity group IV soybean [*Glycine max* (L.) Merr.] Accessions. G3 (Bethesda). 5(11): 2391–403

Ren H, Han J, Wang X, Zhang B et al (2020) QTL mapping of drought tolerance traits in soybean with SLAF sequencing. Crop J 8(6):977–989

Reyna N, Cornelious B, Shannon JG, Sneller CH (2003) Evaluation of a QTL for waterlogging tolerance in Southern soybean germplasm. Crop Sci 43(6):2077–2082

Rhine MD, Stevens G, Shannon G, Wrather A, Sleper D (2010) Yield and nutritional responses to waterlogging of soybean cultivars. Irrig Sci 28:135–142

Riaz A, Kanwal F, Börner A, Pillen K, Dai F, Alqudah AM (2021) Advances in genomics-based breeding of barley: molecular tools and genomic databases. Agronomy 11(5):894

Rizal G, Karki S (2011) Research article alcohol dehydrogenase (ADH) activity in soybean (*Glycine max* [L] Merr) under flooding stress. Electr J Plant Breed 2(1):50–57

Rosenzweig C, Tubiello FN, Goldberg R, Mills E, Bloomfield J (2002) Increased crop damage in the US from excess precipitation under climate change. Global Environ Change 12:197–202

Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90:43–48

Roy SJ, Negrao S, Tester M (2014) Salt resistant crop plants. Curr Opin Biotechnol 26:115–124

Saleem A, Muylle H, Aper J, Ruttink T, Wang J, Yu D, Roldán-Ruiz I (2021) A genome-wide genetic diversity scan reveals multiple signatures of selection in a European soybean collection compared to Chinese collections of wild and cultivated soybean accessions. Front Plant Sci 26(12):631767

Salimath SS, Bhattacharyya MK (1999) Generation of a soybean BAC library, and identification of DNA sequences tightly linked to the Rps1-k disease resistance gene. Theor Appl Genet 98:712–720

Satpute GK, Gupta S, Bhatia VS, Devdas R, Kumawat G, Tiwari VK, Purwar JP, Agrawal A (2019) Multi-tiered selection scheme for breeding drought tolerance in soybean In: Abstr golden jubilee international salinity conference (GJISC): resilient agriculture in saline environments under changing climate: challenges & opportunities, held during February 7–9, 2019 at ICAR–CSSRI. Karnal, Haryana, p 127

Satpute GK, Arya M, Gupta S, Bhatia VS, Ramgopal D, Ratnaparkhe MB, Chandra S, Singh M, Nagar S, Kamble VG, Pandey S, Kumawat G, Shivakumar M, Nataraj V, Rajesh V (2020) Identifying drought tolerant germplasm through multiplexing polygenic traits in soybean (*Glycine max* L Merrill). J Oilseeds Res 37(SI):56–57

Satpute GK, Ratnaparkhe MB, Chandra S, Kamble VG, Kavishwar R, Singh AK, Gupta S, Devdas R, Arya M, Singh M, Sharma MP, Kumawat G, Shivakumar M, Nataraj V, Kuchlan MK, Rajesh V, Srivastava MK, Chitikineni A, Varshney RK, Nguyen HT (2021) Breeding and molecular approaches for evolving drought-tolerant soybeans. In: Giri B, Sharma MP (eds) Plant stress biology. Springer, Singapore, pp 83–130

Sayama T, Nakazaki T, Ishikawa G, Yagasaki K, Yamada N, Hirota N, Hirata K, Yoshikawa T, Saito H, Teraishi M (2009) QTL analysis of seed-flooding tolerance in soybean (*Glycine max* [L] Merr). Plant Sci 176:514–521

Schaefer RJ, Michno JM, Jeffers J, Hoekenga O, Dilkes B, Baxter I, Myers CL (2018) Integrating coexpression networks with GWAS to prioritize causal genes in maize. Plant Cell 30(12):2922–2942

Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J (2010) Genome sequence of the palaeopolyploid soybean. Nature 463:178

Segal DJ, Meckler JF (2013) Genome engineering at the dawn of the golden age. Annu Rev Genomics Hum Genet 14(1):135–158

Seversike TM, Sermons SM, Sinclair TR, Carter TE Jr, Rufty TW (2014) Physiological properties of a drought-resistant wild soybean genotype: transpiration control with soil drying and expression of root morphology. Plant Soil 374(1–2):359–370

Shan Q, Wang Y, Chen K, Liang Z, Li J, Zhang Y, Zhang K, Liu J, Voytas DF, Zheng X, Zhang Y, Gao C (2013) Rapid and efficient gene modification in rice and *Brachypodium* using TALENs. Mol Plant 6(4):1365–1368

Shannon JG, Stevens WE, Wiebold WJ, McGraw RL, Sleper DA, Nguyen HT (2005) Breeding soybeans for improved tolerance to flooding proceedings of the 30th soybean research conference, American Seed Trade Association. Chicago, IL, USA

Shao GH, Song JZ, Liu HL (1986) Preliminary studies on the evaluation of salt tolerance in soybean varieties. Acta Agron Sin 6:30–35

Shao GH, Wan CW, Chang RZ, Chen YW (1993) Preliminary study on the damage of plasma membrane caused by salt stress. Crops 1:39–40

Sharmin RA, Bhuiyan MR, Lv W, Yu Z, Chang F, Kong J, Bhat JA, Zhao T (2020) RNA-Seq based transcriptomic analysis revealed genes associated with seed-flooding tolerance in wild soybean (*Glycine soja* Sieb & Zucc). Environ Exp Bot 171:p103906

Sharma SK, Goloubinoff P, Christen P (2011) Non-native proteins as newly-identified targets of heavy metals and metalloids. In: Banfalvi G (ed) Cellular effects of heavy metals. Springer, pp 263–274

Shi X, Yan L, Yang C et al (2018) Identification of a major quantitative trait locus underlying salt tolerance in 'Jidou 12' soybean cultivar. BMC Res Notes 11:95

Shoemaker RC, Specht JE (1995) Integration of the soybean molecular and classical genetic linkage groups. Crop Sci 35:436–446

Shoemaker RC, Schlueter J, Doyle JJ (2006) Paleopolyploidy and gene duplication in soybean and other legumes. Curr Opin Plant Biol 9:104–109

Shoemaker RC, Grant D, Olson T, Warren WC, Wing R, Yu Y, Kim H, Cregan P, Joseph B, FutrellGriggs M, Nelson W, Davito J, Walker J, Wallis J, Kremitski C, Scheer D, Clifton SW, Graves T, Nguyen H, Wu X, Luo M, Dvorak J, Nelson R, Cannon S, Tomkins J, Schmutz J, Stacey G, Jackson S (2008) Microsatellite discovery from BAC end sequences and genetic mapping to anchor the soybean physical and genetic maps. Genome 51:294–302

Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM, Rock JM, Wu YY, Katibah GE, Zhifang G, McCaskill D, Simpson MA, Blakeslee B, Greenwalt SA, Urnov FD (2009) Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature 459(7245):437–441

Shultz JL, Kazi S, Bashir R, Afzal JA, Lightfoot DA (2007) The development of BAC-end sequence-based microsatellite markers and placement in the physical and genetic maps of soybean. Theor Appl Genet 114:1081–1090

Sigoillot FD, King RW (2010) Vigilance and validation: keys to success in RNAi screening. ACS Chem Biol 6(1):47–60

Sinclair TR, Purcell LC, King CA, Sneller CH, Chen P, Vadez V (2007) Drought tolerance and yield increase of soybean resulting from improved symbiotic N_2 fixation. Field Crop Res 101(1):68–71

Singh RJ, Hymowitz T (1985) An intersubgeneric hybrid between *Glycine tomentella* Hayata and the soybean, *G max* (L) Merr. Euphytica 34:187–192

Singh RJ, Kollipara KP, Hymowitz T (1987) Intersubgeneric hybridization of soybeans with a wild perennial species, *Glycine clandestina* Wendl. Theor Appl Genetics 74:391–396

Singh RJ, Klein TM, Mauvais CJ, Knowlton S, Hymowitz T, Kostow CM (1998a) Cytological characterization of the transgenic soybean. Theor Appl Genet 96:319–324

Singh RJ, Kollipara KP, Hymowitz T (1998b) The genomes of *Glycine canescens* F.J. Herm and *G. tomentella* Hayata of Western Australia and their phylogenetic relationships in the genus *Glycine* Willd. Genome 41:669–679

Singh RJ, Chung GH, Nelson RL (2007) Landmark research in Legumes. Genome 50:525–537

Singh V, Sanwal SK, Kumawat G, Shivakumar M, Satpute GK, Gill BS, Singh Z, Panwar S, Singh J, Sharma PC (2020) Assessing the effect of salt stress on Soybean [*Glycine max* (L) Merrillis] genotypes using AMMI and GGE biplot analysis. J Soil Salinity Water Quality 12(1):95–100

Singleton PW, Bohlool BB (1984) Effect of salinity on nodule formation by soybean. Plant Physiol 74:72–76

Skorupska HT, Shoemaker RC, Warner A, Shipe ER, Bridges WC (1993) Restriction fragment length polymorphism in soybean germplasm of the southern USA. Crop Sci 33:1169–1176

Sloane RJ, Patterson RP, Carter TE Jr (1990) Field drought tolerance of a soybean plant introduction. Crop Sci 30(1):118–123

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Song L, Nguyen N, Deshmukh RK, Patil GB, Prince SJ, Valliyodan B, Mutava R, Pike SM, Gassmann W, Nguyen HT (2016a) Soybean TIP gene family analysis and characterization of GmTIP1; 5 and GmTIP2; 5 water transport activity. Front Plant Sci 7:1564

Song L, Prince S, Valliyodan B et al (2016b) Genome-wide transcriptome analysis of soybean primary root under varying water-deficit conditions. BMC Genomics 17:57

Song Q, Jia G, Zhu Y, Grant D, Nelson RT, Hwang E, Hyten DL, Cregan PB (2010) Abundance of SSR motifs and development of candidate polymorphic SSR markers (BARCSOYSSR_10) in soybean. Crop Sci 50:1950–1960

Song Q, Hyten DL, Jia G, Quigley CV, Fickus EW, Nelson RL, Cregan PB (2013) Development and evaluation of SoySNP50K, a high-density genotyping array for soybean. PLoS One 8(1):e54985

Song Q, Yan L, Quigley C, Fickus E, Wei H, Chen L, Dong F, Araya S, Liu J, Hyten D, Pantalone V, Nelson RL (2020) Soybean BARCSoySNP6K: an assay for soybean genetics and breeding research. Plant J 104(3):800–811

Sosnowski O, Charcosset A, Joets J (2012) BioMercator v3: an upgrade of genetic map compilation and quantitative trait loci meta-analysis algorithms. Bioinformatics 28:2082–2083

Specht J, Hume D, Kumudini SV (1999) Soybean yield potential—a genetic and physiological perspective. Crop Sci 39:1560–1570

Specht JE, Chase K, Macrander M, Graef GL, Chung J, Markwell JP, German M, Orf JH, Lark KG (2001) Soybean response to water: a QTL analysis of drought tolerance. Crop Sci 41(2):493–509

Sreenivasa V, Lal SK, Babu PK, Swamy HKM, Yadav RR, Talukdar A, Rathod DR (2020) Inheritance and mapping of drought tolerance in soybean at seedling stage using bulked segregant analysis. Plant Genet Resour:1–8

Steketee CJ, Schapaugh WT, Carter TE, Li Z (2020) Genome-wide association analyses reveal genomic regions controlling canopy wilting in soybean. G3 Genes Genomes Genetics 10:1413–1425

Stolf-Moreira R, Medri ME, Marcelino FC, de Olivira MLN, Farias JRB, Abdelnoor RV, Nepomuceno AL (2010) Cloning and quantitative expression of drought induced genes in soybean. Genet Mol Res 9:858–867

Staub JE, Serquen FC, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. Hort Sci 31(5):729–739

Suematsu K, Abiko T, Nguyen VL, Mochizuki T (2017) Phenotypic variation in root development of 162 soybean accessions under hypoxia condition at the seedling stage. Plant Prod Sci 20(3):323–335

Sun F, Xu M, Park C, Dwiyanti MS, Nagano AJ, Zhu J, Watanabe S, Kong F, Liu B, Yamada T, Abe J (2019a) Characterization and quantitative trait locus mapping of late-flowering from a Thai soybean cultivar introduced into a photoperiod-insensitive genetic background. PLoS One 14(12):e0226116

Sun TJ, Fan L, Yang J, Cao RZ, Yang CY, Zhang J et al (2019b) A *Glycine max* sodium/hydrogen exchanger enhances salt tolerance through maintaining higher Na+ efflux rate and K+/Na+ ratio in *Arabidopsis*. BMC Plant Biol 19:496

Sun X, Hu Z, Chen R et al (2015) Targeted mutagenesis in soybean using the CRISPR-Cas9 system. Sci Rep 5:10342

Sullivan M, VanToai T, Fausey N, Beuerlein J, Parkinson R, Soboyejo A (2001) Evaluating on-farm flooding impacts on soybean. Crop Sci 41:93–100

Szczepek M, Brondani V, Buchel J, Serrano L, Segal DJ, Cathomen T (2007) Structure-based redesign of the dimerization interface reduces the toxicity of zinc-finger nucleases. Nat Biotechnol 25:786–793

Thao NP, Thu NB, Hoang XL, Van Ha C, Tran LS (2013) Differential expression analysis of a subset of drought-responsive GmNAC genes in two soybean cultivars differing in drought tolerance. Intl J Mol Sci 14:23828–23841

Thibaud-Nissen F, Shealy RT, Khanna A, Vodkin LO (2003) Clustering of microarray data reveals transcript patterns associated with somatic embryogenesis in soybean. Plant Physiol 132(1):118–136

Thompson JA, Nelson RL, Vodkin LO (1998) Identification of diverse soybean germplasm using RAPD markers. Crop Sci 38:1348–1355

Tomkins JP, Mahalingham R, Miller-Smith H, Goicoechea JL, Knapp HT, Wing RA (1999) A soybean bacterial artificial chromosome library for PI 437654 and the identification of clones associated with cyst nematode resistance. Plant Mol Biol 41:25–32

Torkamaneh D, Laroche J, Valliyodan B, O'Donoughue L, Cober E, Rajcan I, Vilela Abdelnoor R, Sreedasyam A, Schmutz J, Nguyen HT, Belzile F (2021) Soybean (*Glycine max*) Haplotype Map (GmHapMap): a universal resource for soybean translational and functional genomics. Plant Biotechnol J 19(2):324–334

Tripathi P, Rabara RC, Reese RN, Miller MA, Rohila JS, Subramanian S et al (2016) A toolbox of genes, proteins, metabolites and promoters for improving drought tolerance in soybean includes the metabolite coumestrol and stomatal development genes. BMC Genomics 17:102

Turner M, Yu O, Subramanian S (2012) Genome organization and characteristics of soybean microRNAs. BMC Genom 13:169

Tuyen DD, Lal SK, Xu DH. Identification of a major QTL allele from wild soybean (*Glycine soja* Sieb. & Zucc.) for increasing alkaline salt tolerance in soybean. Theor Appl Genet 121:229–236

Tuyen DD, Zhang HM, Xu DH (2013) Validation and high-resolution mapping of a major quantitative trait locus for alkaline salt tolerance in soybean using residual heterozygous line. Mol Breed 31:79–86

Vadez V, Kholová J, Hummel G, Zhokhavets U, Gupta SK, Hash CT (2015) LeasyScan: a novel concept combining 3D imaging and lysimetry for high-throughput phenotyping of traits controlling plant water budget. J Exp Bot 66(18):5581–5593

Valliyodan B, Dan Qiu, Patil G, Zeng P, Huang J, Dai L, Chen C, Li Y, Joshi T, Song L, Vuong TD, Musket TA, Xu D, Shannon JG, Shifeng C, Liu X, Nguyen HT (2016) Landscape of genomic diversity and trait discovery in soybean. Sci Rep 31(6):23598

Valliyodan B, Ye H, Song L, Murphy M, Shannon JG, Nguyen HT (2017) Genetic diversity and genomic strategies for improving drought and waterlogging tolerance in soybeans. J Exp Bot 68(8):1835–1849

Valliyodan B, Cannon SB, Bayer PE, Shu S, Brown AV, Ren L, Jenkins J, Chung CY, Chan TF, Daum CG, Plott C, Hastie A, Baruch K, Barry KW, Huang W, Patil G, Varshney RK, Hu H, Batley J, Yuan Y, Song Q, Stupar RM, Goodstein DM, Stacey G, Lam HM, Jackson SA, Schmutz J, Grimwood J, Edwards D, Nguyen HT (2019) Construction and comparison of three reference-quality genome assemblies for soybean. Plant J 100(5):1066–1082

Valliyodan B, Brown AV, Wang J et al (2021) Genetic variation among 481 diverse soybean accessions, inferred from genomic re-sequencing. Sci Data 8:50

VanToai TT, Beuerlein JE, Schmitthenner AF, St Martin SK (1994) Genetic variability for flooding tolerance in soybean. Crop Sci 34:1112–1115

VanToai TT, St Martin SK, Chase K, Boru G, Schnipke V, Schmitthenner AF, Lark KG (2001) Identification of a QTL associated with tolerance of soybean to soil waterlogging. Crop Sci 41:1247–1252

VanToai TT, Hoa TC, Hue TN, Nguyen HT (2010) Flooding tolerance of soybean *[Glycine max* (L) Merr] germplasm from Southeast Asia under field and screen-house environments. The Open Agriculture Journal 4(1):38–46

Vodkin LO, Khanna A, Shealy R, Clough SJ, Gonzalez DO, Philip R, Zabala G, Thibaud-Nissen F, Sidarous M, Stromvik MV, et al (2004) Microarray analysis for global expression constructed with a low redundancy set of 27,500 sequenced cDNAs representing an array of developmental stages and physiological conditions of the soybean plant. BMC Genomics 5:73

Vu HTT, Kilian A, James AT, Bielig LM, Lawn RJ (2015) Use of DArT molecular markers for QTL analysis of drought-stress responses in soybean. II. Marker identification and QTL analyses. Crop Pasture Sci 66:817–830

Wang YH, Zhang XJ, Fan SJ (2015) Genetic diversity of wild soybean populations in Dongying, China, by simple sequence repeat analysis. Genet Mol Res 14(3):11613–11623

Wang LS, Chen QS, Xin DW, Qi ZM, Zhang C, Li SN, Jin Y, Li M, Mei HY, Su AY et al (2018a) Overexpression of *GmBIN2*, a soybean glycogen synthase kinase 3 gene, enhances tolerance to salt and drought in transgenic *Arabidopsis* and soybean hairy roots. J Integr Agri 17:1959–1971

Wang L, Liu L, Ma Y, Li S, Dong S, Zu W (2018b) Transcriptome profiling analysis characterized the gene expression patterns responded to combined drought and heat stresses in soybean. Comput Biol Chem 77:413–429

Wang LX, Guan RX, Li YH, Lin FY, Luan WJ, Li W, Ma YS, Liu ZX, Chang RZ, Qiu LJ (2008a) Genetic diversity of Chinese spring soybean germplasm revealed by SSR markers. Plant Breed 127:56–61

Wang F, Zhao TJ, Yu DY, Chen SY, Gai JY (2008b) Inheritance and QTL analysis of submergence tolerance at seedling stage in soybean. Acta Agron Sin 34:748–753

Wei W, Liang DW, Bian XH, Shen M, Xiao JH, Zhang WK, Ma B, Lin Q, Lv J, Chen X, Chen SY, Zhang JS (2019) GmWRKY54 improves drought tolerance through activating genes in abscisic acid and Ca2+ signaling pathways in transgenic soybean. Plant J 100(2):384–398

Wendt T, Holm PB, Starker CG et al (2013) TAL effector nucleases induce mutations at a pre-selected location in the genome of primary barley transformants. Plant Mol Biol 83:279–285

Wesley SV, Liu Q, Wielopolska A, Ellacott G, Smith N, Singh S, Helliwell C (2003) Custom knock-outs with hairpin RNA-mediated gene silencing. Methods Mol Biol 236:273–286

Wu C, Sun S, Nimmakayala P, Santos FA, Meksem K, Springman R, Ding K, Lightfoot DA, Zhang HB (2004) A BAC- and BIBAC-based physical map of the soybean genome. Genome Res 14:319–326

Wu XL, Zhong GH, Findley SD, Cregan P, Stacey G, Nguyen HT (2008) Genetic marker anchoring by six-dimensional pools for development of a soybean physical map. BMC Genomics 9:28

Wu C, Zeng A, Chen P, Florez-Palacios L, Hummer W, Mokua J, Klepadlo M, Yan L, Ma Q, Cheng Y (2017a) An effective field screening method for flooding tolerance in soybean. Plant Breed 136(5):710–719

Wu C, Chen P, Hummer W, Zeng A, Klepadio M (2017b) Effect of flood stress on Soybean seed germination in the field. Am J Plant Sci 8:53–68

Wu C, Mozzoni LA, Moseley D, Hummer W, Ye H, Chen P, Shannon G, Nguyen H (2020) Genome-wide association mapping of flooding tolerance in soybean. Mol Breed 40(1):1–4

Xie D, Dai Z, Yang Z, Tang Q, Deng C, Xu Y, Wang J, Chen J, Zhao D, Zhang S, Zhang S (2019) Combined genome-wide association analysis and transcriptome sequencing to identify candidate genes for flax seed fatty acid metabolism. Plant Sci 286:98–107

Xie K, Yang Y (2013) RNA-guided genome editing in plants using a CRISPR-Cas system. Mol Plant 6:1975–1983

Xu C, Xia C, Xia Z et al (2018) Physiological and transcriptomic responses of reproductive stage soybean to drought stress. Plant Cell Rep 37:1611–1624

Yamamoto T, Narikawa T (1966) Survey report on cool weather injury in Hokkaido Part III upland crops agriculture, forestry and fisheries research council, ministry of agriculture and forestry. pp 161–180

Yamanaka N, Ninomiya S, Hoshi M, Tsubokura Y, Yano M, Nagamura Y, Sasaki T, Harada K (2001) An informative linkage map of soybean reveals QTLs for flowering time, leaflet morphology and regions of segregation distortion. DNA Res 8:61–72

Yang J, Blanchar RW (1993) Differentiating chloride susceptibility in soybean cultivars. Agron J 85:880–885

Yang W, Wang M, Yue A, Wu J, Li S, Li G, Du W (2014) QTLs and epistasis for drought-tolerant physiological index in soybean (*Glycine max* L.) across different environments. Caryologia 67(1):72–78

Ye H, Song L, Chen H, Valliyodan B, Cheng P, Ali L, Vuong T, Wu C, Orlowski J, Buckley B, Chen P, Shannon JG, Nguyen HT (2018) A major natural genetic variation associated with root system architecture and plasticity improves waterlogging tolerance and yield in soybean. Plant Cell Environ 41(9):2169–2182

Yoo JH, Park CY, Do Heo W, Cheong MS, Park HC, Kim MC, Moon BC, Choi MS, Kang YH, et al (2005) Direct interaction of a divergent *CaM* isoform and the transcription factor, MYB2, enhances salt tolerance in *Arabidopsis.* J Biol Chem 280:3697–3706

Yu Z, Chang F, Lv W, Sharmin RA, Wang Z, Kong J, Bhat JA, Zhao T (2019) Identification of QTN and candidate gene for seed-flooding tolerance in soybean [*Glycine max* (L) Merr] using genome-wide association study (GWAS). Genes 10(12):957

Zeng A, Chen P, Korth K, Hancock F, Pereira A, Brye K, Wu C, Shi A (2017a) Genome-wide association study (GWAS) of salt tolerance in worldwide soybean germplasm lines. Mol Breed 37(3):30

Zeng A, Lara L, Chen P, Luan X, Hancock F, Korth K, Brye K, Pereira A, Wu C (2017b) Quantitative trait loci for chloride tolerance in ‘Osage’ soybean. Crop Sci 57:2345–2353

Zhang J, Wang J, Jiang W, Liu J, Yang S, Gai J, Li Y (2016) Identification and analysis of $NaHCO_3$ stress responsive genes in wild soybean (*Glycine soja*) roots by RNA-seq. Front Plant Sci 7:1842

Zhang M, Liu Y, Cai H, Guo M, Chai M, She Z, Ye L, Cheng Y, Wang B, Qin Y (2020) The bZIP transcription factor GmbZIP15 negatively regulates salt- and drought-stress responses in soybean. Intl J Mol Sci 21(20):7778

Zhang WB, Qiu PC, Jiang HW, Liu CY, Li CD, Hu GH, Chen QS (2012) Dissection of genetic overlap of drought and low-temperature tolerance QTLs at the germination stage using backcross introgression lines in soybean. Mol Biol Rep 39(5):6087–6094

Zhang DY, Kumar M, Xu L et al (2017) Genome-wide identification of major intrinsic proteins in *Glycine soja* and characterization of GmTIP2;1 function under salt and water stress. Sci Rep 7:4106

Zhang W, Liao X, Cui Y, Ma W, Zhang X, Du H, et al (2019) A cation diffusion facilitator, GmCDF1, negatively regulates salt tolerance in soybean. PLoS Genet 15(1):e1007798

Zeng A, Chen P, Korth K, Hancock F, Pereira A, Brye K et al (2017c) Genome-wide association study (GWAS) of salt tolerance in worldwide soybean germplasm lines. Mol Breed 37:30

Zhou QY, Tian AG, Zou HF, Xie ZM, Lei G, Huang J, Wang CM, Wang HW, Zhang JS, Chen SY (2008) Soybean WRKY-type transcription factor genes, GmWRKY13, GmWRKY21, and GmWRKY54, confer differential tolerance to abiotic stresses in transgenic *Arabidopsis* plants. Plant Biotechnol J 6:486–503

Zhou Z, Jiang Y, Wang Z et al (2015) Resequencing 302 wild and cultivated accessions identifies genes related to domestication and improvement in soybean. Nat Biotechnol 33:408–414

Zhu C, Core M, Buckler E, Yu J (2008) Status and prospects of association mapping in plants. Plant Genome 1:5–20

Zhu T, Shi I, Gresshoff P, Keim P (1996) Characterization and application of soybean YACs to molecular cytogenetics. Mol Gen Genet 252:483–488

Zhu YL, Song QJ, Hyten DL, Van Tassell CP, Matukumalli LK, Grimm DR, Hyatt SM, Fickus EW, Young ND, Cregan PB (2003) Single-nucleotide polymorphisms in soybean. Genetics 163:1123–1134

Chapter 2
Designing the Rapeseed Genome for Abiotic Stress Tolerance

Inderpreet Dhaliwal, Indu Rialch, Kusum Rana, Jasmeet Kaur, and Gurpreet Kaur

Abstract *Brassica* species were domesticated as oil producing crops during different periods at many sites throughout the world. Animal fat being pricier, the poor used vegetable oil as a source of their nutrition. Accordingly, world production of vegetable oil has been incremental chiefly due to increased production of soybean, palm and oilseed rape. Rapeseed (*Brassica napus* L.), also known as Canola or Oilseed rape, has thus become an important source of vegetable oil worldwide, and ranks third after soybean and palm. The world population is expected to cross the 9 billion mark by 2050, and to assure food and nutritional security for our soaring future generations, we need to necessarily double the production of food crops by then. However, various environmental stresses negate the realization of this target. Rapeseed thrives very well in countries of the northern hemisphere of the planet having cool and humid climates, making it a very important oil- and protein-crop, since no other crop can produce such high yields of both oil and protein under these climatic conditions. In the coming decades, it has the potential of achieving the rank *numero uno* as the cheapest source of nutritious vegetable oil for the impoverished of the world. Nevertheless, it is prone to various abiotic stresses which not only affect normal growth rate of the plant but also decrease crop productivity by alarming proportions. It is, therefore, imperative to develop new stress tolerant varieties having higher productivity and better adaptation to the abiotic stresses abounding because of climate change. This chapter summarizes the various abiotic stresses afflicting rapeseed; the classical, genetic and molecular approaches that have been employed for breeding for abiotic stress tolerance, together with biotechnological and synthetic biology research breakthroughs aimed at creating abiotic stress-resistant climate-resilient varieties. The combination of classical and

---

I. Dhaliwal (✉) · I. Rialch · K. Rana · J. Kaur · G. Kaur
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, India
e-mail: dhaliwalinderpreet@pau.edu

I. Rialch
e-mail: indurialch@pau.edu

G. Kaur
e-mail: gurpreetkaur@pau.edu

C. Kole (ed.), *Genomic Designing for Abiotic Stress Resistant Oilseed Crops*,
https://doi.org/10.1007/978-3-030-90044-1_2

molecular breeding, being assisted by integrated omics and genome editing breakthroughs, can lead to speed up breeding of the crop and alter the rate of production of rapeseed worldwide, making it feasible to achieve the target of being number one in meeting the demands for vegetable oil of a soaring population.

Keywords *Brassica napus* · Oilseed rape · Rapeseed · Canola · Abiotic stress · Temperature stress · Drought stress · Salt stress

2.1 Introduction

During evolution, all the cultivated crops attained uniformity, but this generated such genetic bottlenecks that nearly all of them now only possess a narrow resilience towards stresses, making them more exposed to abiotic and biotic stresses. Plants regularly endure discrete stresses that hinder their growth and development throughout the growth cycle (Massonnet et al. 2007). Adverse environmental adversities like high and low temperature, mineral toxicity, salt stress, water scarcity and waterlogging, nutrient deficiency etc. constitute the various abiotic stresses that prove damaging for crop production and genome stability (Raza et al. 2019, 2020). It has been anticipated that abiotic stresses can limit global crop production by 70% (Boyer 1982). Drought, salt and temperature stress have a very wide geographical distribution and are the major abiotic determinants that hinder crop productivity. The intensity, time and duration of stress is responsible for plant's susceptible reaction. The situation is further exacerbated due to climate change, with unsuitable ecological circumstances deterring plants from realizing their full genetic potential, thereby leading to decreased crop productivity. Often, abiotic stresses occur in combination and have a severe effect: high temperature and water scarcity frequently occur together during a bout of drought; plants are repeatedly exposed to salt stress, moisture deficit, and frost situations together; drought and mineral toxicities may also occur together.

Brassica species were domesticated as oil producing crops during different periods at many sites throughout the world. Animal fat being pricier, the poor used to rely on vegetable oil as a source of their nutrition. Accordingly, world production of vegetable oil has been incremental chiefly due to increased production of soybean, palm and oilseed rape. Rapeseed, also known as oilseed rape or canola, is cultivated in the temperate climates of the planet during different seasons (Shahzadi et al. 2015; Zhu et al. 2016) chiefly for the extraction of oil from its seed. The oil is utilized for human nutrition as well as a renewable raw material for the chemical industry, as a catch crop for green manuring, as a forage crop and more recently as a source of regenerative energy. Its processing produces a high-protein content meal as a by-product which is used as a high-quality livestock feed. Also known as oil cake, this rapeseed meal is also used as a fertilizer in certain parts of the world.

In world crop production, oilseed crops rank 2nd after cereals and, betwixt them, rapeseed ranks third worldwide with an annual value of 41 billion USD collectively

(USDA 2018). However, it is exposed to many biotic and abiotic stresses which are responsible for reducing its production and productivity. This chapter discusses the various abiotic stresses afflicting oilseed rape; the classical, genetic and molecular approaches employed for breeding stress-resistant crop, together with biotechnological and synthetic biology research breakthroughs, to create abiotic stress-tolerant climate-resilient varieties that are fitter to face the biggest challenge in human history: that of nutritionally feeding 9 billion people by 2050.

2.1.1 *Temperature Stress*

Geographical distribution of plants is predominantly dependent upon a vital climatic factor: temperature (Sutcliffe 1977). Plants can endure temperatures way above the optimum values required for their growth and development; however, above-optimum temperatures cause stress. Crops generally encounter heat stress during the reproductive phase of their development, though cultivated, field-grown crops are under temperature stress for most of their growing season (Mahan et al. 1995). Huge yield losses have been reported because of high temperatures which further get aggravated when combined with losses from other environmental stresses. There are yield differences even between areas with cooler and warmer temperatures or between winter and summer seasons, suggesting the influence high temperature stress has in lowering yields (Paulsen 1994). Plants' response to temperature stress is mainly dependent on the stage of development it is in. Elevated temperatures increase the rate of plant development, thereby shortening reproductive phase (Hall 1992) and lowering the yield potential (Entz and Fowler 1991) through reduction in photosynthetic resources (Morrison 1993). A combination of water and heat stress severely affect the source as well as sink for assimilates (Mendham and Salsbury 1995). In rapeseed, the yield generally relies upon the factors going on before and during flowering stage (Mendham and Salsbury 1995; Angadi et al. 1999).

2.1.2 *Drought Stress*

Water scarcity is one of the most critical environmental stresses that can occur due to low rainfall, salinity, high and low temperatures, and high intensity of light. It is said to be a multidimensional stress that amounts to modifications in the phenological, physiological, morphological, biochemical, and molecular pathways in plants. Drought can critically affect seed germination, plant vegetative growth, seed quality and seed yield. The flowering stage is exceedingly prone to drought injury, inducing seed (29.5%) and oil (31.7%) yield losses (Shekari et al. 2015) in rapeseed. Physiological changes in the water-stressed leaves through osmoregulation and osmotic adjustment of water potential and relative water content have been observed in Brassica crops. Drought stress diminishes leaf size, stem extension and root proliferation, disrupts plant water relations and dwindles the water-use efficiency. Carbon-di-oxide absorption by leaves is slashed primarily due to closure of the stomata, membrane damage and disrupted activity of various enzymes, chiefly those of CO_2 fixation and ATP synthesis. Increased flow of metabolites through the photorespiratory pathway enhances the oxidative load on the tissues, as both mechanisms spawn reactive oxygen species (ROS), the major deterrent to growth.

2.1.3 Salt Stress

Salt stress is the aggregation of extreme salt content in the soil that ultimately inhibits crop growth, leading to death. It is an extremely damaging abiotic stress, causing ionic toxicity, osmotic and oxidative stress simultaneously (Tanveer and Shabala 2018). This can be engendered by alterations in respiration, photosynthesis and metabolism of nucleic acids and proteins (Hasegawa et al. 2000). Extreme salt concentrations adversely hinder cellular homeostasis and several key physiological processes. A direct consequence of salt stress is an incremental build-up of ROS (Hasegawa et al. 2000; Chaves et al. 2009). The soil texture and composition are negatively altered (Vital 2008), and high salt concentrations lead to an imbalance of nutrients and ions, thereby impeding the normal morpho-physiological and biological processes of the plant (Shinwari et al. 1998). This results in higher osmotic pressure of the soil than the root cells, the root cells thus leach out water into the soil instead of absorbing it from the soil, creating water and nutrition imbalance in plants (Sharma et al. 2019). High salt concentration negatively affects seed germination in many Brassica species, resulting in retarded plant growth and development and may even result in the death of the plant under severe conditions (Zamani et al. 2010).

2.1.4 Cold Stress

Low-temperature stress declines both the quality and quantity of yield (Dreccer et al. 2018). The duration and amount of exposure to this stress, stage of development of the plant and moisture content are the key components that determine the extent of injury caused due to frost stress. Frost can suddenly kill the entire crop, especially in areas where temperatures unexpectedly drop below 0 °C. In rapeseed, the whole plant dies if it encounters frost at seedling stage (Shah et al. 2016). It also directly affects flowering and siliqua development and prevents seed formation, thereby affecting crop productivity and causing considerable yield losses (Shah et al. 2016).

2.1.5 Metal Toxicity

The macronutrients (C, O, H, Mg, S, N, Ca, P, and K) and micronutrients (Cu, Zn, Mn, Fe, Mo, B, Ni, Co, Cl, and B) are essential metals required for various physiological and biochemical processes in the plants. The 53 d-block elements that have a density of >5 g/cm^3 are classified as heavy metals (Jarup 2003). Micronutrients copper, zinc, cobalt, nickel and iron are beneficial for better crop growth but if they accumulate in large amount, become harmful (Khan et al. 2018; Narendrula-Kotha et al. 2020), while other metals like arsenate (As), cesium (Cs), lead (Pb), and cadmium (Cd), are toxic even in less amounts (Khalid et al. 2018). Heavy metal accumulation above threshold may disturb soil microbiological equilibrium and thus diminish soil fertility. Crop productivity and quality is adversely affected by heavy metal toxicity (HMT) of soil and water, contaminating swathes of land because of overuse of pesticides, fertilizers, municipal and compost wastes, and heavy metal release from smelting industries and metalliferous mines (Yang et al. 2005). The response to HMT includes decrease in seed germination and growth (Siddiqui et al. 2014), low biomass (Ben Ghnaya et al. 2009), chlorosis (Baryla et al. 2001), antioxidative defense (Nouairi et al. 2009), impaired uptake of essential elements (Feigl et al. 2013), peroxidation of membrane (John et al. 2009) and production of malondialdehyde (Ben Ghnaya et al. 2009).

2.2 Genetic Variation for Abiotic Stress Tolerance

2.2.1 Temperature Stress Tolerance

High temperature during flowering and siliqua filling stage has the most crucial impact on canola yield. Genetic variation for heat tolerance has been reported in cultivated Brassicas. *B. rapa* is least tolerant to thermal stress than *B. napus* and *B. juncea*, whereas, *B. napus* is least to recover from temperature stress (Angadi et al. 2000). Genetic variation for thermo-tolerance during reproductive phase has been observed in different genotypes of *B. napus* (Aksouh-Harradj et al. 2006; Annisa et al. 2013; Koscielny et al. 2018). Traits like pollen fertility, seed set (Young et al. 2004; Wu et al. 2020a, b), vigorous root system (Wu et al. 2018), electrical conductivity, electrical impedance (Wu et al. 2017), chlorophyll fluorescence (Cowley and Luckett 2011) and accumulation of heat shock proteins (Queitsch et al. 2000) can be exploited for breeding of heat tolerant crops.

2.2.2 Drought Tolerance

Being a complex trait, drought tolerance involves an array of component traits. Different traits for drought tolerance have been studied by few workers. Richards and Thurling (1979) reported leaf proline accumulation, leaf chlorophyll stability and germination at low osmotic potentials as criteria for selection. They documented inter-specific variations for heritability of different traits. Maximum broad-sense heritability of 65% for chlorophyll stability in *B. napus*, followed by 55% for germination in *B. rapa* and 40% for proline accumulation in both *B. rapa* and *B. napus* were reported. However, proline accumulation and germination have explicitly lower narrow-sense heritability than broad-sense heritability. Cheema and Sadaqat (2004) reported non-additive, duplicate and complementary epistasis along with significant genotype × environment interactions for yield and its components through generation mean analysis, while evaluating populations under both drought stressed and control conditions. Yang et al. (2008) used diallel crossing design for evaluating the genetic estimates for seed germination under drought conditions. Both general combining ability (GCA) and specific combining ability (SCA) were found to play a significant role for six different germination traits.

2.2.3 Salt Tolerance

Exploration of heritable variation within the available germplasm is pivotal for breeders to extract significant information on various abiotic factors. Inter specific variations have been found for salt tolerance. Amphidiploids of Brassicas are better in salt tolerance than diploid parents (Ashraff 2001). In *B. napus,* cultivar Dunkeld for biomass and seed yield (Qasim 2000), ST9194 for germination (Puppala et al. 1999) and, Rapora, Mytnitskii and Chisayanatane for seed yield (Pokrovskii 1990) have been identified to be tolerant to salt stress conditions. Diallel crosses and generation mean analysis have been utilized to estimate various genetic parameters for traits related to salt tolerance. Additive, non-additive and additive-dominance effects have been found to control genetic variation for traits related to salt tolerance. Rezai and Saeidi (2005) reported both additive and non-additive gene actions for traits related to salt tolerance by utilizing diallel crosses in rapeseed. Additive genetic control has been found for ions such as calcium (Ca), potassium (K), sodium (Na), ratios of different ions (potassium/sodium, calcium/sodium) and stress tolerance index. Qiu and Li (2009) reported the predominance of dominant genes with an additive effect and with little existence of over-dominance for salt tolerance. Additive-dominance effects for salt tolerance traits were reported in *B. napus* (Long et al. 2013), *B. juncea* (Thakral and Prakash 1998) *B. rapa* (Kumar and Yadav 1985) and *B. carinata* (Thakral and Singh 1994).

2.3 Traditional Breeding Approaches for Abiotic Stress Tolerance

2.3.1 Drought Tolerance

Breeding for drought-resistant varieties is one of the best approaches for higher gains in water stress situation. For a complex trait like drought, breeding for its component traits is an ideal approach as compared to breeding per se for drought. Existence of genetic variations for the component traits of drought have already been elucidated. In the past, attempts have been made to breed drought tolerant genotypes in *Brassica* spp. For this, selection for flowering time or harvest index gave better genetic gains than selection for yield performance. The wild species have also been explored to combat drought stress. The wild crucifers, *Sinapsis alba* and *Moricandia arvensis* possess drought tolerance (Warwick 1993). These wild species were utilized for development of intergeneric hybrids with cultivated Brassicas (Bang et al. 2007; Wei et al. 2007). Bang et al. (2007) developed intergeneric hybrids between cultivated diploid *B. oleracea* and *M. arvensis* in an attempt to transfer drought tolerance into cultivated species. Monosomic addition lines of *M. arvensis* in *B. oleracea* have also been generated. Similarly, Wei et al. (2007) developed BC_1 from the cross of *S. alba/B. oleracea//B. oleracea* that had recovered full chromosomal complement of *B. oleracea* with additional chromosomes of *S. alba.*

2.3.2 Salt Tolerance

Enhanced knowledge of biochemical and stress-response signaling pathways has revolutionized the salinity performance of modern varieties. Noteworthy progress has been documented for salt tolerance in wheat, rice and tomato. Some salt tolerant varieties of Indian mustard, viz., CS52, CS54, CS56 have been released for cultivation in India by Central Salinity Research Institute, Karnal. Identification of key component traits and elucidation of mechanism for salt tolerance will help in breeding of salt tolerant varieties. Screening of cultivated germplasm along with wild species can prove beneficial for breeding salt tolerant rapeseed varieties.

2.4 Molecular Breeding Approaches for Abiotic Stress Tolerance

2.4.1 Drought Tolerance

In response to drought stress, biosynthetic and signal transduction pathway of abscisic acid (ABA) has been elucidated in detail (Century et al. 2008). During stress, rapid accumulation of ABA induces stomata closure thereby minimizing losses due to transpiration and protecting the plant from water deficit damage. Farnesyl-transferase (FTA) has been reported as the key negative regulator of ABA sensitivity in the guard cell of stomata. Different subunits (α- or β-subunit) of FTA have been shown to play an important role in *Arabidopsis*. Down-regulation of these subunits affects the stomatal conductance and water transpiration, thus enhancing response to ABA and consequently making the plant tolerate drought (Wang et al. 2005). Transgenics developed with rd29A:anti-*AtFTB* construct also showed resistance to seed abortion under water deficient conditions. Under field trials with normal conditions, yield performance of transgenics was at par with wild-type, while under moderate drought stress conditions, transgenics yielded significantly higher than control. Under drought conditions, lignin accumulation increases, and Caffeic acid O-methyltransferase (COMT) is involved in its synthesis. In *B. napus*, Li et al. (2016) identified 12 homologues of COMT1, denoted as *BnCOMT1-1* to *BnCOMT1-12.* Differential expression studies revealed that under water deficient conditions expression levels of these *BnCOMT1s* decreases. Non-synchronization between the total lignin accumulation processes and *BnCOMT1*s transcripts indicated the involvement of these genes in synthesis of specific subunit of lignin.

Fan et al. (2015) identified two quantitative trait loci (QTLs), *QDT.TxFr.2H* and *QDT.TxFr.5H,* on chromosomes 2H and 5H that showed drought tolerance, while one significant QTL QST.TxFr.7H, located on 7H was found for salinity tolerance in doubled haploid (DH) population of oilseed rape by simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers (Fig. 2.1). Gad et al. (2021) used high density linkage map for mapping of consensus QTLs for germination indices under both control and stress conditions (Fig. 2.2).Thirty-six consensus QTLs across the two study environments and 18 QTLs specific to one study environment affected the drought susceptibility indices of four traits: germination percentage, root length, shoot length and root-to-shoot length ratio and were mapped in *B. napus.* Co-linearity between genetic and physical maps facilitated detection of around 256 candidate genes. Of these, single-nucleotide polymorphisms/insertion–deletion (SNP/InDel) variations were detected in 128 genes and these SNP/InDel could also differentiate the two parents. Some of these, *BnaC03g32780D*, *BnaC03g37030D* and *BnaC09g27300D* are directly related with drought stress tolerance. Li et al. (2014) identified 28, 26 and 31 QTLs for root and shoot related traits under control, water logging and drought conditions, respectively. Mapping of 11 and 19 QTLs related to water logging tolerance coefficients and drought resistance coefficients indicated existence of complex genetic mechanism.

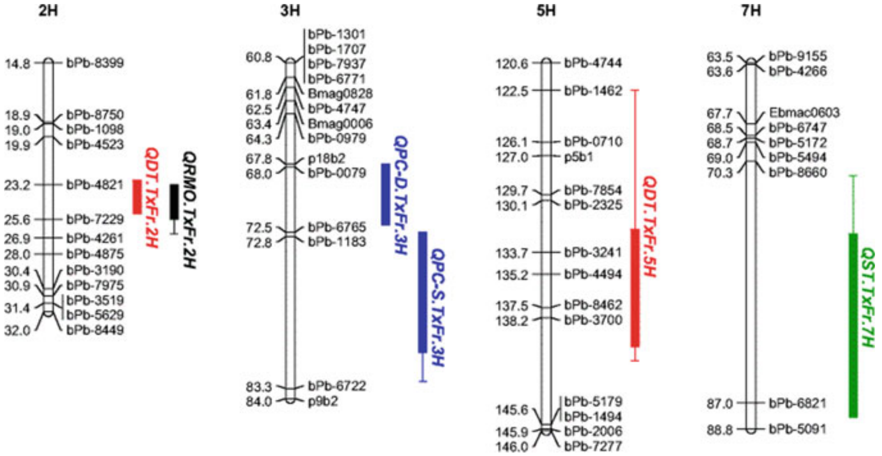

Fig. 2.1 QTL associated with drought tolerance (in red), salinity tolerance (in green), relative moisture content (black) and proline content under drought or salinity stress (in blue) (Adapted from Fan et al. 2015)

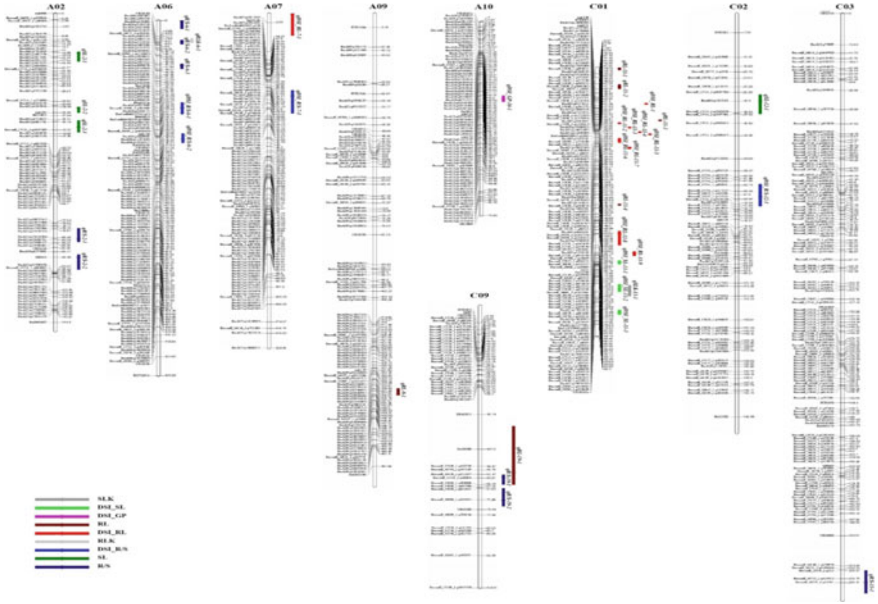

Fig. 2.2 QTL associated with drought tolerance (Adapted from Gad et al. 2021)

However, overlapping of some of the QTLs for both these traits was also suggestive of relatedness of genetic bases controlling these traits.

2.4.2 Salt Stress

Reports on mapping of QTLs for salt stress tolerance in *B. napus* are very scanty. Now a days, transgenic plants are being utilized to test the overexpression of specific up-regulating genes induced by salt stress. Transgenics conferring to salt tolerance in *Brassicas* have also been reported. Besides, the candidate genes for ion homeostasis and osmolyte accumulation have also been documented (Zhang et al. 2004).

Late-embryogenesis abundant (LEA) proteins belonging to hydrophilic protein group provide protection for various abiotic stresses. Dalal et al. (2009) elucidated the functional role of a Group.

4 LEA protein, LEA4-1 in *B. napus.* ABA, salt, cold and osmotic stresses induced the expression of *LEA4-1* gene in leaf tissues whereas constitutive expression was reported in reproductive tissues. Cloning and transformation of the gene in *Escherichia coli* and *Arabidopsis* conferred its role for salt and temperature tolerance. *LEA4-1* gene expressed under both constitutive Cauliflower mosaic virus 35S (*CaMV35S*) as well as Desiccation-responsive 29A (*RD29A*) promoter for abiotic stress and exhibited increase in tolerance to abiotic stresses, particularly salt and drought stresses in *Arabidopsis.* Lang et al. (2017) identified 45 QTLs for salt tolerance that explained 4.80–51.14% of total phenotypic variation. Of these, *qSPAD5*, major QTL for chlorophyll, was mapped on LG5. Fine mapping through Intron polymorphic markers narrowed down its region to 390 kb. This region envisages gene *Bra003640,* a candidate gene for salt tolerance, and spanned a length of 1063 bp with three exons interrupted by two introns. Expression analysis studies for *qSPAD5* significantly differentiated the parents.

2.4.3 Cold/Freezing Stress

In *B. rapa* and *B. napus*, Teutonico et al. (1995) tried to map the QTLs involved in acclimatized freezing tolerance (FTA) and acclimatization ability (FTB). It was however, not possible to map QTLs for FTA in *B. napus.* However, for *B. rapa*, significant QTLs for FTA and FTB were detected on LGs 2, 4, 5 and 7 while two QTLs for non-acclimatized freezing tolerance (FTN) were mapped on LGs 9 and 10. Winter parent contributed positive additive and negative dominance effects for FTN. RFLP probe developed from a cold-induced cDNA in *A. thaliana* mapped near two QTLs for FTA/FTB. Association of RFLP probe with FTA/FTB loci was indicative of their involvement. Kole et al. (2002) identified and compared the QTLs for winter survival (WS), FTA, FTN and FT in *B. rapa* and *B. napus.* For WS, five and 16 QTLs were identified in *B. rapa* and *B. napus* respectively. Of the 16 QTLs in *B.*

napus, six QTLs were stable and were detected for two respective winter seasons. For FTA, 2 and 1 QTLs, respectively, were detected in *B. rapa* and *B. napus.* For FTN, it could be possible to map a single QTL only in *B. rapa.* Correspondence of QTLs for different traits with in a species indicated the involvement of some alleles in providing better acclimatized freezing tolerance. Correspondence of QTLs between *B. rapa* and *B. napus* indicated the presence of allelic variation. Huang et al. (2018) mapped 11 QTLs clustered on seven linkage groups for cold resistance traits in *B. napus.* One QTL, qECYL-4, for electrical conductivity, five QTLs, *qSPADYL-6*, *qSPADYL-7*, *qSPADYS-3, qSPADYS-5* and *qSPADYS-6,* for chlorophyll content, two QTLs, *qMDAYL-3* and *qMDAYS-6* for monoaldehyde and three QTLs, *qSPYL-7, qSPYL-11* and *qSPYL-12* for soluble protein were mapped. Two candidate genes *BnaA08g05330D* and *BnaA08g15470D* encoding the cold-regulated proteins were identified. Differential expression analysis between parents also confirmed their role in resistance to cold.

2.4.4 Boron Deficiency

In *Brassica napus*, Zhang et al. (2014) used a DH population and identified a major novel QTL *qBEC-A3a* on chromosome A03 for boron deficiency (Fig. 2.3).

2.4.5 Phosphorus Deficiency

Phosphorus (P) is a major nutrient affecting growth in the rhizosphere. There are very few reports on underlying genetic mechanisms, QTLs and candidate genes for phosphorus utilization efficiency in Brassicas. Yang et al. (2010) reported the genetic basis of P deficiency tolerance in *B. napus.* Sixty-two significant QTLs were detected for root and associated traits under high and low phosphorus regimes. Of these, three unique QTLs, *uq.A1*, *uq.C3a* and *uq.C3b* were associated directly with root traits and P uptake. Two functional genes, *AtGPT1* and *AtIPS2* related to phosphorus transporters and regulators in Arabidopsis were developed into markers, BnIPS2-C3 and BnGPT1-C3, which were mapped with in the confidence limits of QTLs, *uq.C3a* and *uq.C3b.*

2.5 Post-Genomics Research for Addressing Abiotic Stresses in Rapeseed

The current yield plateau of *Brassica* genus was confronted by various environmental challenges including salinity, extreme temperatures (drought), heat, frost, and heavy

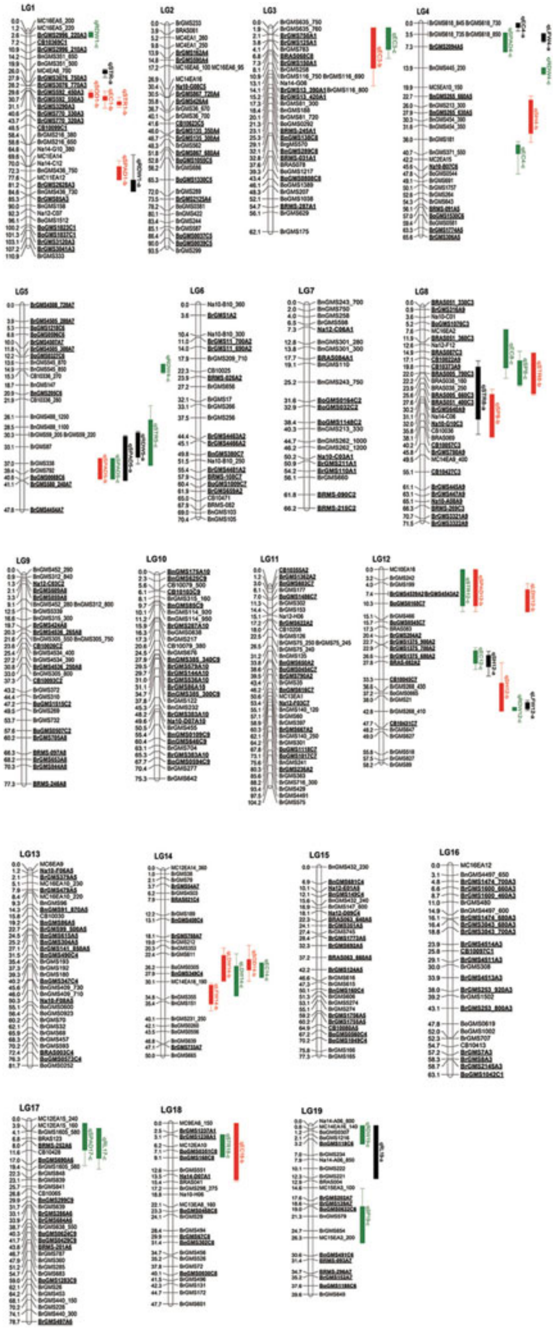

Fig. 2.3 QTLs associated with salt tolerance (Adapted from Lang et al. 2017)

metal stress. The plants are susceptible to salt and high (>30 °C) temperature at seedling stage whereas drought and frost poses major threat during after flowering to grain filling stage (Kumar et al. 2017).

2.5.1 Genomics and Transcriptomics

With the advent of sequencing of five *Brassica* genomes and the development of various omic tools have led to the advancement of Brassica genomics. Nowadays several genomic technologies like high next generation sequencing (NGS) platforms, QTL mapping, genome wide and candidate gene based association mapping (GWAS, CG-AM) based on SSRs, cleaved amplified polymorphic sequence (CAPS), inter simple sequence repeats (ISSRs), SNPs have been implemented to elucidate novel genes and/or markers trait associations against various abiotic stresses (Kole et al 2002; Trick et al. 2009; Wang et al. 2011; Bus et al. 2012; Chalhoub et al. 2014; Bayer et al. 2017; Rahaman et al. 2017; Paritosh et al. 2021). Table 2.1 represents the identification of QTLs in rapeseed against abiotic stress.

Through transcriptomic or RNA profiling researchers have been able to decipher up-regulated and down-regulated genes against abiotic stresses. This was carried out either by next-generation sequencing (NGS), RNA-Seq, expressed sequence tags (ESTs), serial analysis of gene expression (SAGE), and microarrays (Goodwin et al. 2016; Wang et al. 2018; Luo et al. 2019). In *B. napus* Wang et al. (2018) characterized 2167 transcription factors (TFs) from five families including, 518 BnAP2/EREBPs, 252 BnbZIPs, 721 BnMYBs, 398 BnNACs and 278 BnWRKYs against drought, cold, salinity and heat stress, respectively. Out of 2167 TFs nearly 80% TFs were abiotic stress inducible and 315 were differentially expressed genes (DEGs). Comparative transcriptomic analysis by Yong et al. (2014) deciphered 438 transporter genes from 14,719 DEGs in rapeseed roots and leaves under increased salinity stress. Zou et al. (2013) identified 4432 differentially expressed genes under water logging stress in the roots of ZS9 (*B. napus*). After GO enrichment analysis 144 and 191 genes were found to be down-regulated and up-regulated, respectively. Also, Luo et al.

Table 2.1 QTLs identified in rapeseed against various abiotic stresses

S. No.	Type of stress	QTL(s) identified	References
1	Drought and low temperature	31	He et al. (2016)
2	Cold	11	Huang et al. (2018)
3	Salinity	62	Yong et al. (2015)
4	Winter survival and freezing	6, 16	Kole et al (2002)
5	Freezing	4	Asghari et al. (2008)
6	Water logging and drought	26, 31	Li et al. (2014)
7	Salinity	25	Wan et al. (2017)

(2019) characterized 10,233 (up-regulated) and 9111 (down-regulated) DEGs under normal and low temperature conditions in rapeseed genotypes. The up-regulated genes were related with phyto-hormone regulation, signal transduction, and pentose phosphate pathway whereas down-regulated genes were associated with ubiquitin-mediated proteolysis. Likewise, Xin et al. (2019) in rapeseed identified 47,328 DEGs against cold temperatures. Recently Jian et al. (2020) carried out transcriptomic and metabolomics studies in five spring and winter rapeseed lines treated at two temperatures (4 and 28 °C). DEGs of 25,460 and 28,512 and 41 and 47 differentially expressed metabolites each were identified and characterized in both spring and winter types against cold stress response.

2.5.2 Proteomics

Proteomics is the study of total proteins expressed in response to plant stress either in cell wall, organelle, nuclear and phosphor-proteome of an organism (Nakagami et al. 2012; Luan et al. 2018). For proteomics analysis various techniques like yeast two-hybrid (Y2H) system, matrix-assisted laser desorption ionization (MALDI), electro spray ionization (ESI)-MS, and one/two- dimensional gel electrophoresis (2-DGE) were used for proteome profiling against several abiotic stresses (Komatsu et al. 2014; Shao et al. 2015). Proteins like CTRI, CDPK21, TPR, BSL and STN7 are expressed in plants under salt and drought stress (Luo et al. 2015). In rapeseed Yildiz et al. (2015) examined various proteins under salt and lipoic acid stress using 2-DGE and MALDI-TOF MS. A total of 28 proteins have been expressed under stress conditions of which 10 and 18 proteins were expressed during microsporocyte and microspore stage, respectively. Dolatabadi et al. (2019) identified 44 proteins using 2-DGE under salt-stress in rapeseed.

2.5.3 Metabolomics and Phenomics

Metabolomics is study of elucidation of primary and secondary metabolites including organic acids, secondary metabolites, vitamins, amino acids, peptides etcalong with their metabolic and biochemical pathways in any plant species (Razzaq et al. 2019; Raza 2020). Techniques like nuclear magnetic resonance (NMR) and mass spectrometry including liquid chromatography-mass spectrometry (LC–MS), gas chromatography-mass spectrometry (GC–MS), direct injection-mass spectrometry (DIMS), and high-performance liquid chromatography (HPLC) have been used in metabolite profiling (Parida et al. 2018). Nokhrina et al. (2014) overexpressed *Phosphatidyl inositol-specific phospholipas*e (*PLC2*) gene in rapeseed and evaluated metabolome of transgenic plants. The transgenic plants showed increased level of maltose, unsaturated free fatty acids, glycerol, glycerol 3-phosphate, raffinose, stachyose under low temperatures. Jian et al. (2020) identified 41 and 47 metabolites in spring and winter rapeseed ecotypes respectively under cold stress. In addition to metabolomics, plant phenomics or high throughput phenotyping (HTP) has been used in various crop improvement studies such as GWAS, CG-AM, genomic selection (GS), marker assisted selection (MAS), and QTL mapping. Plant phenomics is the analysis of plants morphology in a specific environmental using various techniques. The recent advancements in HTP tools including X-ray tomography, hyper spectral imaging, and visible light imaging has led to phenotyping evaluation of thousands of plants at a time (Raza et al. 2021). For HTP, several phenotyping centers like National Plant Phenomics Center (UK), Phenome UK, Nordic Plant Phenotyping Network (Denmark), PHENOPSIS-INRA (France) have been setup across the world (Raza et al. 2021). Very few examples of HTP are available in rapeseed (Zhang et al. 2017; Li et al. 2020).

2.5.4 Bioinformatics

With the advent of genome sequencing of *Brassica* species, numerous bioinformatics software's and databases have been hugely developed for various gene annotations, prediction, domain and motif analysis, sequence alignment, phylogenetic studies, transcriptomic analysis, de novo assembling, plant-pathogen interaction studies etc. Table 2.2 summarizes some available databases in public domain as well as other software's along with URLs.

2.6 Genetic Engineering for Abiotic Stress Tolerance

The molecular characterization and functional validation of several identified genes against various abiotic stresses in rapeseed through recent omic technologies have paved the way to elucidate host-pest interactions and to characterize the gene function. The functional validation was carried out by Knock in or Knock down or Knock out strategies using RNA interference (RNAi), host induced gene silencing (HIGS), virus induced gene silencing (VIGS), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated proteins (Cas) system (Yin and Hulbert 2015; Majumdar et al. 2017; Ma et al. 2019). Transgenics and/or genetic engineering against abiotic stress resistance include the manipulation or incorporation of certain gene(s) that are responsible for stress regulation, and improvise plant growth under extreme environmental conditions. Literature cites several transgenics developed in *Brassica* species with improved abiotic stress tolerance. For example, Tabasum (2016) introduced *PDH45 DNA helicase* gene from pea in to rapeseed. The 53 transformed *B. napus* transgenic lines showed resistance against salt, drought and heat stress. The other successful examples of Brassica transgenics along with transferred genes against various abiotic stresses are listed in Table 2.3.

Table 2.2 Summary of some available databases and softwares along with their URLs

S. No.	Software	URL	References
1	Brassica genome	http://brassicagenome.net/databases.php	Stein et al. (2002)
2	Phytozome	http://www.phytozome.net	Goodstein et al. (2012) Nordberg et al. (2014)
3	PlantGDB	http://www.plantgdb.org	Dong et al. (2004) Duvick et al. (2008)
4	Ensembl Plants	http://plants.ensembl.org	Bolser et al. (2016)
5	TAGdb	http://www.plantgdb.org/TaGDB/cgi-bin/blastGDB.pl	Marshall et al. (2010)
6	KEGG	http://www.genome.jp/kegg/genome/plant.html	Kanehisa and Goto (2000)
7	Genevestigator	http://genevestigator.com	Hruz et al (2008)
8	BRAD	http://brassicadb.org/brad/	Cheng et al. (2011)
9	PRGdb	http://prgdb.org/prgdb/	Sanseverino et al. (2010)
10	UniProt	http://www.uniprot.org	The UniProt consortium
11	SMART	http://smart.embl-heidelberg.de/	Schultz et al. (2000)
12	Cutadapt 1.7.1	https://pypi.python.org/pypi/cutadapt	Martin (2011)
13	DEGseq	R-package	Wang and Wang (2020)
14	DAVID	https://david.ncifcrf.gov/	Huang et al. (2009a, b)
15	MEGA 6.0	https://www.megasoftware.net/	Tamura et al. (2013)
16	agriGO	http://bioinfo.cau.edu.cn/agriGO/	Tian et al. (2017)
17	Patho Plant	http://www.pathoplant.de/	Bolívar et al. (2014)
18	SWISS-2D-PAGE WORLD-2D-PAGE	https://world-2dpage.expasy.org/swiss-2dpage/	

Table 2.3 The transgenics developed in rapeseed along with transferred genes against various abiotic stresses

S. No.	Gene(s)	Stress resistance	References
1	*AtTrx-h2*	Salinity	Kim et al. (2017)
2	*CBF1/2/3*, *BnCBF5* and *BnCBF17*	Freezing tolerance	Moustafa et al. (2016)
3	*YHem1*	Salinity	Lu et al. (2013)
4	*AtDWF4*	Heat and drought	Sun et al. (2015), Sahni et al. (2016)
5	*BnPLC2*	Low temperature	Smolikova et al. (2016)
6	DREB, *AtPLD-α-1*	Salinity and drought	Qamarunnisa et al. (2015) Kong et al. (2011)
7	*BnKCS1-1*, *BnKCS1-2*, and *BnCER1-2*	Drought tolerance	Wang et al. (2020)

Table 2.4 Genome editing using CRISPR/Cas9 system in rapeseed against abiotic stress and agronomic traits

S. No.	Gene(s)	Trait	References
Abiotic stresses			
1	*BnLLA10*	Salt tolerance	Hu et al. (2018)
2	*BnaA6.RGA,* DELLA protein	Transcription factor for drought tolerance	Wu et al. (2020a)
Other agronomic traits			
3	*BnSPL3-Cnn, BnSPL3-C4, BnSPL3-C3, BnSPL3-A4, BnSPL3-A5, JAGGED*	Pod development and shattering	Zaman et al. (2019)
4	*BnaMAX1*	Plant architecture and yield	Zheng et al. (2020)
5	*CLAVATA*	Silique development	Yang et al. (2018)
6	*FAD2*	Oil quality	Okuzaki et al. (2018)

2.7 Genome Editing

CRISPR/Cas technology has inadvertently revolutionized the concept of genome editing in crop plants (Raza et al. 2021). Recently, this technology has been widely used in gene editing of various abiotic stresses in *Brassica* species. Table 2.4 summarizes successful studies using CRISPR/Cas9 system in *B. napus*.

2.8 Future Perspectives

Conventional plant breeding has been unsuccessful in contributing towards a 100% tolerance/resistance towards abiotic stresses so far. Climate change coupled with agronomic significance of rapeseed in global agriculture call for advanced techniques to combat various abiotic stresses in plants. Drought, salt, frost and high temperature stresses significantly affect the crops' productivity, making it imperative to develop/identify abiotic stress (drought/salt/cold/heat) tolerant cultivars. The missing links in the metabolic pathways and genes involved in stress response need to be elucidated to solve the puzzle. An encouraging research direction is genome editing via the CRISPR/Cas system. Engineering metabolic pathways and stress-associated genes for climate-resilient cultivar development can open up new vistas of research in the crop. Speed breeding has emerged as a new time-saving breeding tool and the combination of omics, genome editing, and speed breeding can accelerate rapeseed production with improved traits and increased abiotic stress tolerance. Synthetic biology, another emerging approach, can be applied in conjunction with engineering methods to develop climate-smart rapeseed plants.

References

Aksouh-Harradj NM, Campbell LC, Mailer RJ (2006) Canola response to high and moderately high temperature stresses during seed maturation. Can J Plant Sci 86(4):967–980

Angadi SV, Cutforth HW, Miller PR, McConkey B (1999) Effect of high temperature stress on yield and yield components of three Brassica species. In: Soils and crops workshop

Angadi SV, Cutforth HW, Miller PR, McConkey BG, Entz MH, Brandt SA, Volkmar KM (2000) Response of three Brassica species to high temperature stress during reproductive growth. Can J Plant Sci 80:693–701

Annisa CS, Turner NC, Cowling WA (2013) Genetic variation for heat tolerance during the reproductive phase in *Brassica rapa.* J Agron Crop Sci 199:424–435

Asghari A, Mohammadi S, Moghaddam M, Toorchi M, Mohammadinasab AD (2008) Analysis of quantitative trait loci associated with freezing tolerance in rapeseed (*Brassica napus* L.). Biotechnol Biotechnol Equip 22(1):548–552

Ashraf M (2001) Relationships between growth and gas exchange characteristics in some salt-tolerant amphidiploid Brassica species in relation to their diploid parents. Env Exp Bot 45:155–163

Bang SW, Sugihara K, Jeung BH, Kaneko R, Satake E, Kaneko Y, Matsuzawa Y (2007) Production and characterization of intergeneric hybrids between *Brassica oleracea* and a wild relative *Moricandia arvensis.* Plant Breed 126:101–103

Baryla A, Carrier P, Franck F, Coulomb C, Sahut C, Havaux M (2001) Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. Planta 212:696–709

Bayer PE, Hurgobin B, Golicz AA, Chan CK, Yuan Y, Lee H, Renton M, Meng J, Li R, Long Y, Zou J (2017) Assembly and comparison of two closely related *Brassica napus* genomes. Plant Biotechnol J 15(12):1602–1610

Ben Ghnaya A, Charles G, Hourmant A, Ben Hamida J, Branchard M (2009) Physiological behaviour of four rapeseed cultivars (*Brassica napus* L.) submitted to metal stress. Crit Rev Biol 332:363–370

Bolívar JC, Machens F, Brill Y, Romanov A, Bülow L, Hehl R (2014) 'In silico expression analysis', a novel PathoPlant web-tool to identify abiotic and biotic stress conditions associated with specific cis-regulatory sequences. Database (Oxford)

Bolser D, Staines DM, Pritchard E, Kersey P (2016) Ensemble plants: integrating tools for visualizing, mining, and analyzing plant genomics data. Methods Mol Biol 1374:115–140

Boyer JS (1982) Plant productivity and environment. Science 218:443–448

Bus A, Hecht J, Huettel B, Reinhardt R, Stich B (2012) High-throughput polymorphism detection and genotyping in *Brassica napus*using next-generation RAD sequencing. BMC Genomics 13(1):1–11

Century K, Reuber TL, Ratcliffe OJ (2008) Regulating the regulators: the future prospects for transcription-factor-based agricultural biotechnology products. Plant Physiol 147:20–29

Chalhoub B, Denoeud F, Liu S, Parkin IA, Tang H, Wang X, Chiquet J, Belcram H, Tong C, Samans B (2014) Early allopolyploid evolution in the post-neolithic *Brassica napus* oilseed genome. Science 345:950–953

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms. Ann Bot 103:551–560

Cheema KL, Sadaqat HA (2004) Potential and genetic basis of drought tolerance in canola (*Brassica napus*): I. Generation mean analysis for some phenological and yield components. Intl J Agric Biol 6:74–81

Cheng F, Liu S, Wu J, Fang L, Sun S et al (2011) BRAD, the genetics and genomics database for Brassica plants. BMC Plant Biol 11:136

Couto R, Comin JJ, Souza M, Ricachenevsky FK, Lana M, Gatiboni L (2018) Should heavy metals be monitored in foods derived from soils fertilized with animal waste? Front Plant Sci 9:732

Cowley R, Luckett D (2011) Chlorophyll fluorescence as a method to detect moisture limiting stress in canola. In: 17th Australian research assembly on brassicas (ARAB)

Dalal M, Tayal D, Chinnusamy V, Bansal KC (2009) Abiotic stress and ABA-inducible Group 4 LEA from Brassica napus plays a key role in salt and drought tolerance. J Biotechnol 139(2):137–145

Dolatabadi N, Toorchi M, Valizadeh M, Bandehagh A (2019) The proteome response of salt-sensitive rapeseed (*Brassica napus* L.) genotype to salt stress. Not Bot Horti Agrobot Cluj-Napoca 47(1):17–23

Dong Q, Schlueter SD, Brendel V (2004) PlantGDB, plant genome database and analysis tools. Nucleic Acids Res 32 (Database issue):D354–D359. https://doi.org/10.1093/nar/gkh046

Dreccer MF, Fainges J, Whish J, Ogbonnaya FC, Sadras VO (2018) Comparison of sensitive stages of wheat, barley, canola, chickpea and field pea to temperature and water stress across Australia. Agric Meteorol 248:275–294

Duvick J, Fu A, Muppirala U, Sabharwal M, Wilkerson MD, Lawrence CJ, Lushbough C, Brendel V (2008) PlantGDB: a resource for comparative plant genomics. Nucleic Acids Res 36(suppl_1):D959–D965

Entz MH, Fowler DB (1991) Agronomic performance of winter versus spring wheat. Agron J 83:527–532

Fan Y, Shabala S, Ma Y, Xu R, Zhou M (2015) Using QTL mapping to investigate the relationships between abiotic stress tolerance (drought and salinity) and agronomic and physiological traits. BMC Genomics 16(1):1–1

Feigl G, Kumar D, Lehotai N, Tugyi N, Molnár Á, Ördög A, Szepesi Á, Gémes K, Laskay G, Erdei L, Kolbert Z (2013) Physiological and morphological responses of the root system of Indian mustard (*Brassica juncea* L. Czern.) and rapeseed (*Brassica napus* L.) to copper stress. Ecotoxicol Environ Saf 94:179–189

Gad M, Chao H, Li H, Zhao W, Lu G, Li M (2021) QTL mapping for seed germination response to drought stress in *Brassica napus*. Front Plant Sci 11:2276

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res 40:1178–1186

Goodwin S, McPherson JD, McCombie WR (2016) Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet 17(6):333

Hall AE (1992) Breeding for heat tolerance. Plant Breed Rev 10:129–168

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Biol 51(1):463–499

He Y, Mao S, Gao Y, Zhu L, Wu D, Cui Y, Li J, Qian W, Li M (2016) Genome-wide identification and expression analysis of WRKY transcription factors under multiple stresses in *Brassica napus*. PLoS One 11(6):e0157558

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinformat. https://doi.org/10.1155/2008/420747

Hu L, Zhang H, Yang Q, Meng Q, Han S, Nwafor CC, Khan MHU, Fan C, Zhou Y (2018) Promoter variations in a homeobox gene, BnA10.LMI1, determine lobed leaves in rapeseed (*Brassica napus*L.). Theor Appl Genet 131(12):2699–2708

Huang DW, Sherman BT, Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37(1):1–13

Huang DW, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4(1):44–57

Huang Z, Zhao N, Qin M, Xu A (2018) Mapping of quantitative trait loci related to cold resistance in *Brassica napus* L. J Plant Physiol 231:147–154

Jarup L (2003) Hazards of heavy metal contamination. Br Med Bull 68:167–182

Jian H, Xie L, Wang Y, Cao Y, Wan M, Lv D, Li J, Lu K, Xu X, Liu L (2020) Characterization of cold stress responses in different rapeseed ecotypes based on metabolomics and transcriptomics analyses. Peer J 8:e8704

John R, Ahmad P, Gadgil K, Sharma S (2009) Cadmium and lead-induced changes in lipid peroxidation, antioxidative enzymes and metal accumulation in *Brassica juncea* L. At three different growth stages. Arch Agric Soil Sci 55:395–405

Jorrin-Novo JV, Komatsu S, Sanchez-Lucas R, de Francisco LER (2019) Gel electrophoresis-based plant proteomics: past, present, and future. Happy 10th Anniv J Proteom! J Proteom 198:1–10

Kanehisa M, Goto S (2000) KEGG: Kyoto Encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30

Khalid S, Shahid M, Bibi I, Sarwar T, Shah A, Niazi N (2018) A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. Intl J Environ Res 15:895

Khan ZI, Ugulu I, Sahira S, Ahmad K, Ashfaq A, Mehmood N (2018) Determination of toxic metals in fruits of *Abelmoschus esculentus* grown in contaminated soils with different irrigation sources by spectroscopic method. Intl J Environ Res 12:503–511

Kholghi M, Toorchi M, Bandehagh A, Ostendorp A, Ostendorp S, Hanhart P, Kehr J (2019) Comparative proteomic analysis of salt-responsive proteins in canola roots by 2-DE and MALDI-TOF MS. Biochim Biophys Acta Proteins Proteom 1867(3):227–236

Kim J, Lee WJ, Vu TT, Jeong CY, Hong S-WS-W, Lee H (2017) High accumulation of anthocyanins via the ectopic expression of AtDFR confers significant salt stress tolerance in *Brassica napus* L. Plant Cell Rep 36(8):1215–1224

Kole C, Thormann CE, Karlsson BH, Palta JP, Gaffney P, Yandell B, Osborn TC (2002) Comparative mapping of loci controlling winter survival and related traits in oilseed *Brassica rapa* and *B. napus.* Mol Breed 9(3):201–210

Komatsu S, Kamal AH, Hossain Z (2014) Wheat proteomics: proteome modulation and abiotic stress acclimation. Front Plant Sci 5:684

Kong F, Mao S, Du K, Wu M, Zhou X, Chu C, Wang Y (2011) Comparative proteomics analysis of OsNAS1 transgenic *Brassica napus* under salt stress. Chin Sci Bull 56(22):2343–2350

Koscielny CB, Hazebroek J, Duncan RW (2018) Phenotypic and metabolic variation among spring Brassica napus genotypes during heat stress. Crop Pasture Sci 69:284–295

Kumar D, Yadav IS (1985) Line × tester analysis for certain quantitative traits in brown sarson (B. campestris var. brown sarson) on normal and alkaline soils. Soc Adv Breed Res Asia Ocea J 17:21–28

Kumar S, Prasad R, Singh D, Yadav SP, Kumar V (2017) Screening of Brassica germplasm and breeding material against *Erysiphe cruciferarum* causing powdery mildew of rapeseed mustard under artificial condition. Environ Ecol 35:112–115

Lang L, Xu A, Ding J, Zhang Y, Zhao N, Tian Z, Liu Y, Wang Y, Liu X, Liang F, Zhang B (2017) Quantitative trait locus mapping of salt tolerance and identification of salt-tolerant genes in *Brassica napus* L. Front Plant Sci 8:1000

Li H, Feng H, Guo C, Yang S, Huang W, Xiong X, Liu J et al (2020) High-throughput phenotyping accelerates the dissection of the dynamic genetic architecture of plant growth and yield improvement in rapeseed. Plant Biotechnol J 18(11):2345–2353

Li W, Lu J, Lu K, Yuan J, Huang J, Du H, Li J (2016) Cloning and phylogenetic analysis of *Brassica napus* L. Caffeic acid O-Methyltransferase 1 gene family and its expression pattern under drought stress. PLoS One 11(11):e0165975

Li Z, Mei S, Mei Z, Liu X, Fu T, Zhou G, Tu J (2014) Mapping of QTL associated with waterlogging tolerance and drought resistance during the seedling stage in oilseed rape (*Brassica napus*). Euphytica 197(3):341–353

Long WH, Pu HM, Zhang JF, Qi CK, Zhang XK (2013) Screening of *Brassica napus*for salinity tolerance at germination stage. Chin J Oil Crop Sci 35:271–275

Lopes MS, Reynolds MP (2012) Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. J Exp Bot 63:3789–3798

Lu S, Bahn SC, Qu G, Qin H, Hong Y, Xu Q, Zhou Y, Hong Y, Wang X (2013) Increased expression of phospholipase Dα1 in guard cells decreases water loss with improved seed production under drought in *Brassica napus*. Plant Biotechnol J 11(3):380–389

Luan H, Shen H, Pan Y, Guo B, Lv C, Xu R (2018) Elucidating the hypoxic stress response in barley (Hordeumvulgare L.) during waterlogging: a proteomics approach. Sci Rep 8:1–13

Luo J, Tang S, Peng X, Yan X, Zeng X, Li J, Li X, Wu G, Yang D (2015) Elucidation of cross-talk and specificity of early response mechanisms to salt and PEG-simulated drought stresses in Brassica napus using comparative proteomic analysis. PLoS One 10(10):e0138974

Luo T, Xian M, Zhang C, Hu L, Xu Z (2019) Associating transcriptional regulation for rapid germination of rapeseed (*Brassica napus*L.) under low temperature stress through weighted gene co-expression network analysis. Sci Rep 9:1–16

Ma C, Zhu C, Zheng M, Liu M et al (2019) CRISPR/Cas9-mediated multiple gene editing in *Brassica oleracea* var. *capitata* using the endogenous tRNA-processing system. Hortic Res 6:20

Mahan JR, McMicheal BL, Wanjura DF (1995) Methods for reducing the adverse effects of temperature stress on plants: a review. Environ Exp Bot 35:251–258

Majumdar R, Rajasekaran K, Cary JW (2017) RNA Interference (RNAi) as a potential tool for control of mycotoxin contamination in crop plants: concepts and considerations. Front Plant Sci 8:200

Marshall DJ, Hayward A, Eales D et al (2010) Targeted identification of genomic regions using TAGdb. Plant Method 6:19

Martin M (2011) Cutadapt removes adapter sequences from high throughput sequencing reads. Embnet J 17:10–12

Massonnet C, Costes E, Rambal S, Dreyer E, Regnard JL (2007) Stomatal regulation of photosynthesis in apple leaves: Evidence for different water-use strategies between two cultivars. Ann Bot 100:1347–1356

Mendham NJ, Salsbury PA (1995) Physiology, crop development, growth and yield. In: Kimber DS, McGregor DI (eds) Brassica oilseeds: production and utilization. CAB International, London, pp 11–64

Morrison MJ (1993) Heat stress during reproduction in summer rape. Can J Bot 71:303–308

Moustafa K, Cross JM (2016) Genetic approaches to study plant responses to environmental stresses: an overview. Biology 5(2):20

Nakagami H, Sugiyama N, Ishihama Y, Shirasu K (2012) Shotguns in the front line: phosphoproteomics in plants. Plant Cell Physiol 53:118–124

Narendrula-KothaR TG, Mehes-Smith M, Kalubi K, Nkongolo K (2020) Metal toxicity and resistance in plants and microorganisms in terrestrial ecosystems. Rev Environ Contam T 249:1–27

Nokhrina K, Ray H, Bock C, Georges F (2014) Metabolomic shifts in Brassica napus lines with enhanced BnPLC2 expression impact their response to low temperature stress and plant pathogens. GM Crops Food 5(2):120–131

Nordberg H, Cantor M, Dusheyko S, Hua S, Poliakov A, Shabalov I, Smirnova T, Grigoriev IV, Dubchak I (2014) The genome portal of the department of energy joint genome institute: updates. Nucleic Acids Res 42(1):26–31

Nouairi I, Ben Ammar W, Ben Youssef N, Ben Miled DD, Ghorbal M, Zarrouk M (2009) Antioxidant defense system in leaves of Indian mustard (*Brassica juncea*) and rape (*Brassica napus*) under cadmium stress. Acta Physiol Plant 31:237–247

Okuzaki A, Ogawa T, Koizuka C, Kaneko K, Inaba M, Imamura J, Koizuka N (2018) CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in *Brassica napus*. Plant Physiol Biochem 131:63–69

Parida AK, Panda A, Rangani J (2018) Metabolomics-guided elucidation of abiotic stress tolerance mechanisms in plants. Plant metabolites and regulation under environmental stress. Elsevier, Amsterdam, The Netherlands, pp 89–131

Paritosh K, Yadava SK, Singh P, Bhayana L, Mukhopadhyay A et al (2021) A chromosome-scale assembly of allotetraploid*Brassica juncea* (AABB) elucidates comparative architecture of the A and B genomes. Plant Biotecnol J 19(3):602–614

Paulsen GM (1994) High temperature responses of crop plants. In: Boote KJ, Bennett JM, Sinclair TR, Paulsen GM (eds) Physiology and determination of crop yield, ASA, CSSA, SSSA, Madison, WI, pp 365–389

Pokrovskii VB (1990) Promising forms of winter swede rape. Selektsiya-iSemenovodstvo Moskva 4:24–25

Puppala N, Fowler JL, Poindexter L, Bhardwaj HL (1999) Evaluation of salinity tolerance of canola germination. In: Janick J (ed) Perspectives on new crops and new uses. ASHS Press, Alexandria, VA, pp 251–253

Qamarunnisa S, Jamil I, Raza S, Azhar A, Naqvi SM (2015) Genetic improvement of canola against abiotic stress through incorporation of DREB gene. Asian J Agric Biol 3:77–104

Qasim M (2000) Physiological and biochemical studies in a potential oilseed crop Canola (*Brassica napus* L.) under salinity (NaCl) stress. Ph.D thesis, Department of Botany, University of Agriculture, Faisalabad, Pakistan

Qiu Y, Li XX (2009) Genetic analysis of salinity tolerance in *Brassica campestris* L. China Vegetab 1:21–25

Queitsch C, Hong SW, Vierling E, Lindquist S (2000) Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. Plant Cell 12(4):479–492

Rahaman M, Mamidi S, Rahman M (2017) Association mapping of agronomic traits of canola (*Brassica napus*L.) subject to heat stress under field conditions. Aust J Crop Sci 11(09):1094–1105

Raza A (2020) Metabolomics: a systems biology approach for enhancing heat stress tolerance in plants. Plant Cell Rep. https://doi.org/10.1007/s00299-020-02635-8

Raza A, Ashraf F, Zou X, Zhang X, Tosif H (2020) Plant adaptation and tolerance to environmental stresses: mechanisms and perspectives. In: Hasanuzzaman M (ed) Plant ecophysiology and adaptation under climate change: mechanisms and perspectives I. Springer, Singapore, pp 117–145

Raza A, Razzaq A, Mehmood SS, Hussain MA, Wei S, He H, Zaman QU, Xuekun Z, Hasanuzzaman M (2021) Omics: the way forward to enhance abiotic stress tolerance in *Brassica napus* L. GM Crops Food 12(1):251–281

Raza A, Razzaq A, Mehmood SS, Zou X, Zhang X, Lv Y, Xu J (2019) Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. Plants 8:34

Razzaq A, Sadia B, Raza A, Khalid Hameed M, Saleem F (2019) Metabolomics: a way forward for crop improvement. Metabolites 9:303

Rezai AM, Saeidi G (2005) Genetic analysis of salt tolerance in early growth stages of rapeseed (*Brassica napus* L.) genotypes. Indian J Genet 65:269–273

Richards RA, Thurling N (1979) Genetic analysis of drought stress response in rapeseed (*Brassica campestris* and *B. napus*) III. Physiological characters. Euphytica 28:755–759

Sahni S, Prasad BD, Liu Q, Grbic V, Sharpe A, Singh SP, Krishna P (2016) Overexpression of the brassinosteroid biosynthetic gene DWF4 in *Brassica napus* simultaneously increases seed yield and stress tolerance. Sci Rep 6:28298

Sanseverino W, Roma G, De Simone M, Faino L, Melito S, Stupka E, Frusciante L, Ercolano MR (2010) PRGdb: a bioinformatics platform for plant resistance gene analysis. Nucleic Acids Res 38:814–821

Schultz J, Copley RR, Doerks T, Ponting CP, Bork P (2000) SMART: a web-based tool for the study of genetically mobile domains. Nucleic Acids Res 28(1):231–234

Shah SH, Ali S, Hussain Z, Jan SA, Din JU, Ali GM (2016) Genetic improvement of tomato (*Solanum lycopersicum*) with AtDREB1A gene for cold stress tolerance using optimized Agrobacterium mediated transformation system. Intl J Agric Biol 18:471–482

Shahzadi T, Khan FA, Zafar F, Ismail A, Amin E, Riaz S (2015) An overview of Brassica species for crop improvement. AmerEuras J Agric Environ Sci 15(1568):1573

Shao S, Guo T, Aebersold R (2015) Mass spectrometry-based proteomic quest for diabetes biomarkers. Biochimica Biophysica Acta (BBA) Proteins Proteom 1854:519–527

Sharma P, Kannu P, Sardana V, Choudhary OP, Banga SS (2019) Physiological and biochemical basis of salinity tolerance in Indian mustard (*B. juncea*). 15th International Rapeseed Congress, Berlin, June 16–19, Abstract number: 171

Shekari F, Soltaniband V, Javanmard A, Abbasi A (2015) The impact of drought stress at different stages of development on water relations, stomatal density and quality changes of rapeseed (*Brassica napus* L.). Iran Agric Res 34(2):81–90

Shinwari ZK, Nakashima K, Miura S, Kasuga M, Seki M, Yamaguchi-Shinozaki K, Shinozaki K (1998) An Arabidopsis gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. Biochem Biophys Res Commun 250(1):161–170

Siddiqui MM, Abbasi BH, Ahmad N, Ali M, Mahmood T (2014) Toxic effects of heavy metals (Cd, Cr and Pb) on seed germination and growth and DPPH-scavenging activity in *Brassicarapa* var Turnip. Toxicolind Health 30:238–249

Smolikova G, Shavarda A, Alekseichuk I, Chantseva V, Medvedev S (2016) Themetabolomic approach to the assessment of cultivar specificity of *Brassica napus* L. seeds. Russ J Genet Appl Res 6:78–83

Song JM, Liu DX, Xie WZ, Yang Z, Guo L, Liu K, Yang QY, Chen LL (2020) BnPIR: *Brassica napus* pangenome information resource for 1,689 accessions. Plant Biotechnol J 19:412–414

Stein LD, Mungall C, Shu S et al (2002) The generic genome browser: a building block for a model organism system database. Genome Res 12:1599–1610

Sun X, Feng X, Li C, Zhang Z, Wang L (2015) Study on salt tolerance with YHem1 transgenic canola (*Brassica napus*). Physiol Plant 154:223–242

Sutcliffe JF (1977) Plants and temperature. E Arnold, London, p 57

Tabasum A (2016) Enhancement of abiotic stress tolerance in Canola (*Brassica napus*) through genetic engineering approach. PhD Thesis. https://minervaaccess.unimelb.edu.au/handle/11343/113595

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Tanveer M, Shabala S (2018) Targeting redox regulatory mechanisms for salinity stress tolerance in crops. In: Kumar V, Wani S, Suprasanna P, Tran LS (eds) Salinity responses and tolerance in plants, vol 1. Springer. Cham, Switzerland, pp 213–234

Teutonico RA, Yandell B, Satagopan JM, Ferreira ME, Palta JP, Osborn TC (1995) Genetic analysis and mapping of genes controlling freezing tolerance in oilseed Brassica. Mol Breed 1(4):329–339

Thakral NK, Prakash K (1998) Combining ability for vigour characters under normal and saline environments in Indian mustard [*Brassica juncea* (L.) Czern.&Coss.]. J Oilseed Res 15:234–237

Thakral NK, Singh H (1994) Combining ability for vigour character sunder normal and saline environment in Ethiopian mustard. Ann Arid Zone 33:245–247

The UniProt Consortium (2021) UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res 49:D1

Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z (2017) agriGO v2. 0: a GO analysis toolkit for the agricultural community, 2017 update. Nucleic Acids Res 45(W1):W122–W129

Trick M, Long Y, Meng J, Bancroft I (2009) Single nucleotide polymorphism (SNP) discovery in the polyploid*Brassica napus*using Solexatranscriptome sequencing. Plant Biotechnol J 7:334–346

USDA FAS (2018) Oilseeds: world markets and trade (Foreign Agricultural Service/USDA). Available from: https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf

Vital SA, Fowler RW, Virgen A, Gossett DR, Banks SW, Rodriguez J (2008) Opposing roles for superoxide and nitric oxide in the NaCl stress induce regulation of antioxidant enzyme activity in cotton callus tissue. Environ Exp Bot 62:60–68

Wan H, Chen L, Guo J, Li Q, Wen J, Yi B, Ma C, Tu J, Fu T, Shen J (2017) Genome-wide association study reveals the genetic architecture underlying salt tolerance-related traits in rapeseed (*Brassica napus* L.). Front Plant Sci 8:593

Wang L, Wang X (2020) DEGseq: identify differentially expressed genes from RNA-seq data. R package version 1.44.0 (Bioconductor 3.12)

Wang P, Yang C, Chen H, Luo L, Leng Q, Li S, Han Z, Li X, Song C, Zhang X (2018) Exploring transcription factors reveals crucial members and regulatory networks involved in different abiotic stresses in *Brassica napus*L. BMC Plant Biol 18:1–21

Wang X, Wang H, Wang J, Wu J, Liu S et al (2011) The genome of the mesopolyploid crop species *Brassica rapa.* Nat Genet 43:1035–1039

Wang Y, Beaith M, Chalifoux M, Ying J, Uchacz T, Sarvas C, Griffiths R, Kuzma M, Wan J, Huang Y (2009) Shoot-specific down-regulation of protein farnesyltransferase (α-subunit) for yield protection against drought in canola. Mol Plant 2:191–200

Wang Y, Jin S, Xu Y, Li S, Zhang S, Yuan Z, Li J, Ni Y (2020) Overexpression of BnKCS1-1, BnKCS1-2, and BnCER1-2 promotes cuticular wax production and increases drought tolerance in *Brassica napus*. Crop J 8:26–37

Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan J, Dennis DT et al (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J 43:413–424

Warwick SI (1993) Wild species in the tribe *Brassicaceae* (*Cruciferae*) as sources of agronomic traits. In: Guide to the wildgermplasm of *Brassica* and allied crops. Technical Bulletin 17E, 1–19. Center for Land and Biological Resources Research Branch, Agriculture Canada, Ottawa, Ontario, Canada

Wei WH, Zhang SF, Wang LJ, Li J, Chen B, Wang Z, Luo LX, Fang XP (2007) Cytogenetic analysis of F1, F2 and BC1 plants from intergeneric sexual hybridization between *Sinapis alba* and *Brassica oleracea* by genomic in situ hybridization. Plant Breed 126:392–398

Wu GZ, Shi QM, Niu Y, Xing MQ, Xue HW (2007) Shanghai RAPESEED database: a resource for functional genomics studies of seed development and fatty acid metabolism of *Brassica.* Nucleic Acids Res 36:D1044–D1047

Wu J, Yan G, Duan Z, Wang Z, Kang C, Guo L, Liu K, Tu J, Shen J, Yi B, Fu T (2020a) Roles of the *Brassica napus* DELLA protein BnaA6.RGA, in modulating drought tolerance by interacting with the ABA signaling component BnaA10.ABF2. Front Plant Sci 11:577

Wu W, Duncan RW, Ma BL (2017) Quantification of canola root morphological traits under heat and drought stresses with electrical measurements. Plant Soil 415(1):229–244

Wu W, Ma BL, Whalen JK (2018) Enhancing rapeseed tolerance to heat and drought stresses in a changing climate: perspectives for stress adaptation from root system architecture. Adv Agron 151:87–157

Wu W, Shah F, Duncan RW, Ma BL (2020) Grain yield, root growth habit and lodging of eight oilseed rape genotypes in response to a short period of heat stress during flowering. Agric For Meteorol 287:107954

Xin H, Xianchao N, Pan X, Wei L, Min Y, Yu K, Lunwen Q, Wei H (2019) Comparative transcriptome analyses revealed conserved and novel responses to cold and freezing stress in *Brassica napus* L. G3: Genes Genomes Genet 9:2723–2737

Yang CJ, Cheng Y, Zou CS, Zhang XK, Zheng PY, Li GY, Yang C (2008) Combining ability and genetic effects of germination ability in different *Brassica napus* L. cultivar under simulated drought stress. Acta Agron Sin 34:1744–1749

Yang M, Ding G, Shi L, Feng J, Xu F, Meng J (2010) Quantitative trait loci for root morphology in response to low phosphorus stress in *Brassica napus.* Theor Appl Genet 121(1):181–193

Yang X, Feng Y, He Z, Stoffell PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353

Yang Y, Zhu K, Li H, Han S, Meng Q (2018) Precise editing of CLAVATA genes in *Brassica napus* L. regulates multilocularsilique development. Plant Biotechnol J 16:1322–1335

Yıldız M, Akçalı N, Terzi H (2015) Proteomic and biochemical responses of canola (*Brassica napus* L.) exposed to salinity stress and exogenous lipoic acid. J Plant Physiol 179:90–99

Yin C, Hulbert S (2015) Host induced gene silencing (HIGS), a promising strategy for developing disease resistant crops. Gene Technol 4:130

Yong HY, Wang C, Bancroft I, Li F, Wu X, Kitashiba H, Nishio T (2015) Identification of a gene controlling variation in the salt tolerance of rapeseed (*Brassica napus* L.). Planta 242:313–326

Yong HY, Zou Z, Kok EP, Kwan BH, Chow K, Nasu S, Nanzyo M, Kitashiba H, Nishio T (2014) Comparative transcriptome analysis of leaves and roots in response to sudden increase in salinity in *Brassica napus*by RNA-seq. Biomed Res Intl 2014:467395

Young LW, Wilen RW, Bonham-Smith PC (2004) High temperature stress of Brassica napus during flowering reduces micro- and megagametophyte fertility, induces fruit abortion, and disrupts seed production. J Exp Bot 55:485–495

Zaman QU, Chu W, Hao M, Shi Y, Sun M, Sang SF, Mei D, Cheng H, Liu J, Li C, Hu Q (2019) CRISPR/Cas9-mediated multiplex genome editing of JAGGED gene in *Brassica napus* L. Biomolecules 9:725

Zamani Z, Nezami MT, Habibi D, Khorshidi MB (2010) Effect of quantitative and qualitative performance of four canola cultivars (*Brassica napus*L.) to salinity conditions. Adv Environ Biol 4(3):422–427

Zhang D, Hua Y, Wang X, Zhao H, Shi L, Xu F (2014) A high-density genetic map identifies a novel major QTL for boron efficiency in oilseed rape (*Brassica napus* L.). PLoS One 9(11):e112089

Zhang JZ, Creelman RA, Zhu JK (2004) From laboratory to field. Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. Plant Physiol 135:615–621

Zhang X, Huang C, Wu D, Qiao F, Li W, Duan L, Wang K, Xiao Y, Chen G, Liu Q (2017) High-throughput phenotyping and QTL mapping reveals the genetic architecture of maize plant growth. Plant Physiol 173:1554–1564

Zheng M, Zhang L, Tang M, Liu J, Liu H, Yang H, Fan S (2020) Knockout of two *BnaMAX1* homologs by CRISPR/ Cas9-targeted mutagenesis improves plant architecture and increases yield in rapeseed (*Brassica napus* L.). Plant Biotechnol J 18:644–654

Zhu M, Monroe JG, Suhail Y, Villiers F, Mullen J, Pater D, Hauser F, Jeon BW, Bader JS, Kwak JM, Schroeder JI (2016) Molecular and systems approaches towards drought-tolerant canola crops. New Phytol 210(4):1169–1189

Zou X, Tan X, Hu C, Zeng L, Lu G, Fu G, Cheng Y, Zhang X (2013) The transcriptome of *Brassica napus* L. roots under waterlogging at the seedling stage. Intl J MolSci 14:2637–2651

Chapter 3

Sunflower and Abiotic Stress: Genetics and Breeding for Resistance in the—Omics Era

Sunflower Abiotic Stress Breeding

Nada Hladni, Chao-Chien Jan, Milan Jocković, Sandra Cvejić, Siniša Jocić, Aleksandra Radanović, and Dragana Miladinović

Abstract Sunflower is considered more tolerant and adaptable to abiotic stresses than many other crops. It can be successfully grown in marginal soils and in semi-arid conditions. Hence, sunflower crop stands a very good chance of surviving a changing environment with its broad genetic base and the use of emerging—omics technologies. In the future, it will be necessary to improve sunflower crop productivity under different abiotic stresses occurring individually or simultaneously Future work in sunflower genetics and breeding for abiotic stress tolerance should be directed to the research and exploitation of the available genetic resources, as useful sources of genes for resilience. An integrated multidisciplinary approach based on genetics and—omics, physiology, and modelling, along with the application of modern breeding tools, should be used for designing of novel sunflower varieties, more resilient for abiotic stresses and extreme environmental conditions that are becoming more frequent due to climate change. In this chapter, we present a review on how sunflower could be adapted to abiotic stresses, respective traits,

---

N. Hladni (✉) · M. Jocković · S. Cvejić · S. Jocić · A. Radanović · D. Miladinović
Institute of Field and Vegetable Crops, Novi Sad, Serbia
e-mail: nada.hladni@ifvcns.ns.ac.rs

M. Jocković
e-mail: milan.jockovic@ifvcns.ns.ac.rs

S. Cvejić
e-mail: sandra.cvejic@ifvcns.ns.ac.rs

S. Jocić
e-mail: sinisa.jocic@ifvcns.ns.ac.rs

A. Radanović
e-mail: aleksandra.dimitrijevic@ifvcns.ns.ac.rs

D. Miladinović
e-mail: dragana.miladinovic@ifvcns.ns.ac.rs

C.-C. Jan
Retired, USDA, Agricultural Research Service, Northern Crop Science Laboratory,
Edward T. Schafer Agricultural Research Center, Fargo, ND 58102, USA

C. Kole (ed.), *Genomic Designing for Abiotic Stress Resistant Oilseed Crops*,
https://doi.org/10.1007/978-3-030-90044-1_3

genetic resources, and tools for their introduction into the cultivated sunflower, thus making sunflower tolerant to the extreme climatic conditions.

Keywords *Helianthus annuus* · Abiotic stress · Breeding · Tolerance · Genomic selection · Multidisciplinary approach

3.1 Introduction

Cultivated sunflower (*Helianthus annuus* L.) is a globally important oilseed crop, mostly used for oil production from seed, but it is also used as a protein crop for human consumption, as well as for feed (Hladni and Miladinović 2019). It is cultivated on over 26.5 million hectares in more than 60 countries (FAO 2017), with the production that takes the fourth place among oilseeds, after soybean, oil palm, and oilseed rape (Jocić et al. 2015; Kaya et al. 2015). Sunflower oil is of a very high quality and generally sells for a premium price compared to soybean, rapeseed, cottonseed, and groundnut. Depending on the breeding goals and final use, we could say that there are three basic sunflower types: oilseed, confectionery, and ornamental sunflower (Seiler and Jan 2010).

Because of its specific structure of its main organs (root, stem, leaves, and head), sunflower is successfully grown in quite a few countries on, so-called, marginal soils. In most casses, in semiarid conditions where almost every year an abiotic stress of one kind or another is present acting as a limiting factor on crop production (Škorić 2009). Hence, sunflower crop stands a very good chance of adapting to changes in the environment, with its broad genetic base and the emerging technologies that will allow traits and gene mining from the vast and relatively unexploited wild crop relatives' gene pool and has been proposed as a potential model crop for adaptation to a changing environment (Seiler 2018). Further understanding of the underlying physiology of the genotype-specific responses of sunflower to predictable and unpredictable environmental variation could enhance the efficiency of the selection for improved stress tolerance and allow cultivation of this crop across an even larger area (de la Vega and Chapman 2010; Miladinović et al. 2019).

In this chapter we present a review on possibilities for sunflower adaptation to abiotic stress, respective traits, genetic resources, and tools for their introduction into the cultivated sunflower, thus making sunflower even more tolerant to the extreme climatic conditions (Fig. 3.1).

3.2 Abiotic Stress Tolerance Related Traits

In breeding for tolerance to abiotic stresses, the basic task is to reduce their adverse effects of on sunflower growth and yield. Characterization of abiotic stress tolerance and traits related to it is very complex and interrelated to many factors. As a

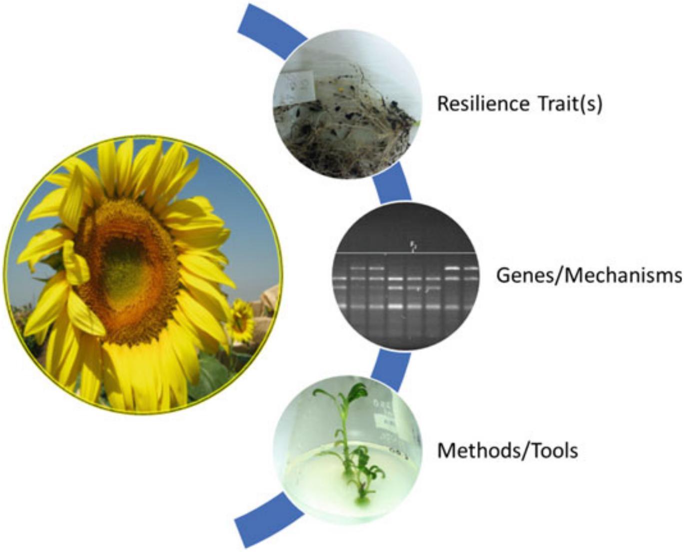

Fig. 3.1 Designing of abiotic-stress-tolerant sunflower: Traits, mechanisms and tools

crop frequently grown on marginal soils and in less extensive production systems, sunflower faces many abiotic stresses, thus requiring study of different abiotic stress-related traits, with the aim of their pyramiding and combining in one resilient genotype (Table 3.1).

3.2.1 Root Characters

In the past decades, breeders were mostly occupied with improvements of above-ground parts of sunflower plant neglecting one of perhaps the most important parts of the plant for its ability to adapt to different environmental conditions. Because of that, it is necessary to focus future breeding efforts on improvement root traits, as well, in order to minimize the effect of stressful conditions on plant development. These efforts could be facilitated by novel phenotyping platforms for non-invasive root analysis, that enable efficient characterization of root architecture and investigation of the developmental dynamics and root growth (Radanović et al. 2018).

Kaya (2016) stated that in sunflower, drought tolerant genotypes should be attributed with powerful roots in order to increase water uptake from the soil. Cultivated sunflower has the potential to trap soil moisture reserves that are inaccessible to many other crops and root traits. Root length and diameter, root length density and root volume, along with fresh and dry root weight and total dry matter are a significant indicator of sunflower tolerance to root drought (Song et al. 1999; Rauf 2008; Comas et al. 2013). However, there is a lack of useful data about morphological

Table 3.1 Traits related to abiotic stress tolerance in sunflower

Abiotic stress	Trait(s)	References
Root characters	Root length and diameter Root length density Root volume Fresh and dry root weight	Rauf (2008), Nagarathna et al. (2012), Comas et al. (2013)
Heat tolerance	Increased transpiration Tolerance to intensive transpiration Deep and powerful root system Total number of nodes Reduced leaf temperature Leaf and head orientation at the time of anthesis Pollen viability under heat stress	Rauf et al. (2012), Seiler (2012), Škorić (2012), Kalyar et al. (2013b, 2014), Razaq et al. (2017)
Cold tolerance	Chlorophyll content Chlorophyll fluorescence Specific leaf area	Allinne et al. (2009), Hniličková et al. (2017)
Drought tolerance	Tolerance to high osmotic pressure Reduced transpiration Chlorophyll fluorescence Photosynthetic performance index Root weight Cuticular wax Leaf cuticular transpiration Leaf temperature in early phases Leaf pubescence Altered leaf angle Leaf rolling Stay-green	Miller (1987), Škorić (1989, 1992, 2009), Belhassen et al. (1996), Panković (1996), Parameswaran (1996), Vranceanu (2000), Chimenti et al. (2004), Petcu et al. (2008), Onemli and Gucer (2010), Sato et al. (2012), Kulundžić et al. (2016), Umar and Siddiqui (2018), Cicek et al. (2019)
Flooding and submergence tolerance	Anaerobic energy production Hypocotyl diameter Chlorophyll concentration Leaf mass per area	Wample and Reid (1978), Torres and Diedenhofen (1981), Lenssen et al. (2004), Gao et al. (2019)
Salinity tolerance	Proline accumulation Germination index Germination vigour index Dry matter accumulation Relative growth rate Relative growth reduction Root growth Shoot fresh weight Leaf ion content Plant height	Hussain and Rehman (1993), Prakash et al. (1993), Singh (2000), Ashraf and Harris (2004), Lexer et al. (2004), Fernandez-Martinez et al. (2009), Seiler (2012), Škorić (2012), Li et al. (2020), Wenhui et al. (2020)

(continued)

Table 3.1 (continued)

Abiotic stress	Trait(s)	References
Water use efficiency	Quantum yield of photosynthesis II Photosynthetic efficacy index Relationship between WUE and Δ13C isotope Temperature of the leaves	Lambrides et al. (2004), Canavar et al. (2014), Kulundžić et al. (2016)

and physiological root parameters of sunflower that can be useful in breeding as it requires the knowledge about root traits and their effect on sunflower productivity while it is known that depth-efficient roots for more water uptake are one of indicators of physiological drought tolerance (Comas et al. 2013; Kaya 2016). Ongoing climate drift in recent years motivated scientists for thorough evaluation of plant performance under stress conditions including detailed physiological studies as well as genomic analyses with special attention that has been given to evaluate relationship between root-related traits and performance under water limitation (Mitchell et al. 2013; Mickelbart et al. 2015; Kusmec et al. 2017; Scoffoni et al. 2017a, b; Li et al. 2018). Results from several studies gave clearer picture about transformations in root morphology under water deficit and it has been noticed that plants often increase the distribution of root biomass and change root morphology under water restriction (Pace et al. 1999; Uga et al. 2011; Tardieu 2013; Comas et al. 2013). Rauf et al. (2009) evaluated sunflower root characteristics under different water regimes and results indicated that drought had repressive effect on root weight and shoot length while elevate effect on root length and root-to-shoot ratio.

Biotechnological tools increased the progress in identification of the genetic basis of root traits variations in sunflower. Quantitative trait loci (QTL) associated with increased root foraging, root length, root biomass, lateral root length, and root angle have been identified (MacMillan et al. 2006; Courtois et al. 2009; Ruta et al. 2010; Uga et al. 2011, 2013; Mace et al. 2012; Christopher et al. 2013; El-Soda et al. 2014; Gao and Lynch 2016). Using genome wide association (GWA) study Masalia et al. (2018) measured several root and growth traits in sunflower in well-watered and water limited environments and identified 13 genomic regions that were associated with the traits of interest across the two environments. The authors found that water limitation reduced seedling size and produced a shift toward deeper rooting and also discovered evidence of pleiotropy across multiple traits, as well as numerous environmentally independent genetic effects. They also suggested that the majority of alleles associated with these traits have consistent effects across environments.

3.2.2 Heat Tolerance

One of the main abiotic factors which, especially in recent years, has become threatening to sunflower production, is the stress caused by high temperatures. Heat stress is defined as the high temperature period lasting enough to cause significant yield reduction and has become one of the major abiotic stresses that occupies parts of Asia, America, some parts of Europe and the whole Africa and Australia (Singh 2004a; Kalyar et al. 2013a). Negative impact of heat stress on molecular, cellular, physiological, phenological, and agronomic traits of sunflower is sunflower is reflected in the decrease of seed number per head, rate and duration of seed and embryo growth, seed weight and change in seed oil characteristics (Chiementi et al. 2002; Prasad and Staggenborg 2008; Kalyar et al. 2013a). Higher air temperature can have a negative affect on sunflower growth by inducing shorter developmental stages as well as lead to early senescence and diminish oxidative protection in sunflower primary leaves (De la Haba et al. 2014). Terzić et al. (2017) found that temperatures above 27 °C reduce nectar production, while those above 33 °C completely stop nectar production. Sunflower can also adapt to higher temperatures by increasing transpiration rate, which keeps the leaves relatively cool. This mechanism is also correlated with high yield due to a positive correlation of transpiration with photosynthetic rate and achene yield (Kalyar et al. 2013a).

It is possible to achieve heat tolerance in sunflower by avoiding adverse conditions or by breeding varieties and species with increased resistance to heat shocks. Different management practices can be applied in order minimize the negative impact of heat stress, such as earlier sowing date, that is found to lead to a significant increase in the leaf area and to enable sunflower plants to avoid water and heat stress during flowering initiation (Barros et al. 2004). Screening available germplasm is the first step to choose adequate source for heat tolerance for the use in breeding. Wild sunflower relatives present valuable source for many important traits in this regard (Lexer et al. 2003; El Midaoui et al. 2003; Warburton et al. 2017; Seiler et al. 2017). Essential criteria in breeding sunflower for tolerance to high temperatures and heat stress, are deep and well-developed root system, tolerance to intensive transpiration, increased pollination capacity, high seed filling rate and rapid synthesis of oil under hot conditions (Seiler 2012; Škorić 2012). Negative effect of heat stress can also be reduced with selection on proper head, leaf, or petiole inclination (Kalyar et al. 2013b).

3.2.3 Cold Tolerance

One of the limiting abiotic factors faced by many plant species are low temperatures which can occur at different stages of the development. In sunflower, each genotype has an optimal range of temperatures for its normal growth and development (Škorić 2009). Considering that usual sowing date in the northern hemisphere is the end of March and the beginning of April, sunflower is faced with the low temperatures at early stages of the development such as germination, emergence, and the 2–3 leaf stage, especially in early plantings and during the maturation period of sunflower production at higher altitudes. Gornik (2011) studied the effect of different temperature treatments on sunflower seed in order to increase its tolerance to chilling and concluded that dry seed storage at 5 °C reduced seedling injuries exhibited by external root discoloration and increased chlorophyll content in leaves. Tetreault et al. (2016) evaluated low temperature tolerance among three natural populations of the perennial sunflower species *Helianthus maximiliani* and found that freezing tolerance was the highest in plants from the northernmost latitude under both non-cold-acclimated and cold-acclimated experimental conditions. Furthermore, authors concluded that plants from all three populations retained the ability to increase freezing tolerance through the process of cold acclimation. In order to better understand sunflower tolerance to low temperatures Hniličkova et al. (2017) conducted study in order to identify the physiological mechanisms associated with the resistance and tolerance of young sunflower plants to freezing temperatures and found that there were no significant changes of osmotic potential in a reaction to the effect of freezing temperatures. However, the knowledge on frost tolerance of sunflower plants after exposure to a period of low temperature is still poorly understood, especially on molecular basis. Hewezi et al. (2006) reported about initial characterization of the transcriptome activity of sunflower and found that that the down-regulation and/or non-induction of genes having a critical role in tolerance to low temperature.

3.2.4 Drought Tolerance

Drought is one of the biggest challenges to crop production in the twenty-first century as well as sunflower production worldwide, severely reducing yield, oil content, oil quality, and other important yield traits (Tyagi et al. 2018; Hladni et al. 2018a, b). Considering that temperature level has increased during vegetation period for most field crops, the biggest concern is crop production in arid regions as higher evaporation will cause increase in water deficit (Homann 2017). Drought stress in sunflower cause many physiological changes as it starts with diminishing of plant water content, then leaf water potential, biomass, cell enlargement and growth and with closing stomata, mainly due to turgor loss (Javaid et al. 2015). In a comprehensive study of Keipp et al. (2020) authors concluded that oil content in sunflower seed was not reduced by drought stress and that reduced oil yield was a result of lower seed weight, limited by a decreased cell diameter. Consequently, different management strategies are applied in practice in order to minimize the damage caused by drought, such as moving the sowing date in order to escape drought period or foliar application of abscisic acid and potassium chloride (Škorić 2012; Hussain et al. 2013).

During domestication process cultivated sunflower lost some drought survival mechanisms found in wild relatives (Seiler et al. 2017). In breeding for drought tolerance, the most widely used *Helianthus* species are *H. argophyllus* and *H. anomalus* (Baldini and Vanozzi 1999; Seiler 2007). Among wild relatives *Helianthus anomalus* is distinguished with large achene and relatively high oil content which makes it desirable in breeding purposes and was identified as a target species, particularly for abiotic stress tolerance and adaptation to extreme soil properties (Seiler et al. 2006; Seiler 2007; Kantar et al. 2015). Several other sunflower wild relatives, widely adapted to drought conditions, can be used as a potential source for tolerant genes such as *H. mollis*, *H. deserticola*, *H. hirsutus*, *H. maximiliani*, *H. tuberosus* etc. (Škorić 2009; Vassilevska-Ivanova et al. 2014).

One of the main objectives in many sunflower breeding programs is tolerance to drought and therefore it is important to identify the physiological, metabolic and molecular mechanisms underlying the response of plants to drought stress. Development of lines and hybrids that are drought-tolerant in sunflower breeding implies thorough study of the relationship between drought tolerance traits and yield and effective screening methods for these traits. Screening genotypes by measuring photosynthetic performance index and leaf temperature in early phases of development can be used to classify responses of genotypes to water deficit (Kulundžić et al. 2016; Cicek et al. 2019). In sunflower breeding for drought tolerance, it is necessary to improve efficiency of genotypes to use available water. Water use efficiency is positively correlated with improved harvest index which is defined as seed yield per total above-ground biomass (Hütsch and Schubert 2017; Keipp et al. 2019, 2020). According to study of Onemly and Gucer (2010) one of the best selection criteria for drought tolerance at early vegetative stage are number of leaves and root weight. Newer study from Pekcan et al. (2016) evaluated effects of drought stress on sunflower stems and roots and indicated that dry and total fresh weight, as well as total root fresh weight are important indicators for drought tolerance. Drought indexes as a quantitative measure of several variables should be used in order to more efficient and precise study drought tolerance (Razzaq et al. 2017).

3.2.5 Flooding and Submergence Tolerance

Abiotic stress caused by changes in water availability can be either water deficit caused by drought period, or excess in water availability causing water logging/submergence due to flooding period (Pradhan and Mohanty 2013; Mustroph 2018). Excessive water negatively affects productivity and viability in plants (Tamang and Fukao 2015). Hence, water logging/submergence is one of the most important abiotic stresses in agricultural crop production which affects 10% of the land area worldwide (Conaty et al. 2008). Being a complex stress, flooding restricts gas diffusion between the plant, soil and atmosphere (Van Dongen and Licausi 2015; Voesenek and Bailey-Serres 2015). In such unfavorable conditions, insufficient or complete absence of oxygen in plant cell is restricting mitochondria activity and together with restricted carbon dioxide (CO_2) in leaves cause energy crisis within plant cell (Mustroph 2018). Earlier studies on effect of varying periods of flooding of sunflower roots demonstrated different changes in shoot morphology such as leaf epinasty, death of shoot apex, extreme chlorosis and hypertrophy of nodes and internodes (Wample and Reid 1978). Sunflower sensitivity to water logging is logging is also outlined in a newer study of Grassini et al. (2007) who concluded that water logging during grain filling determines direct physiological responses such as reduced plant leaf area and leaf capacity to fix carbon and negative effect on plant capacity to absorb water. Negative effect of excess water stress on sunflower plants during the sowing-emergence period is also described by Loose et al. (2017) who reports that water stress substantially reduces emergence, plant density, shoot and root growth, even after 48-h stress. Authors also concluded that water excess also causes morphological changes such as leads to the formation of adventitious and secondary roots. Anatomical adaptations such as aerenchyma formation, the formation of a barrier against radial oxygen loss, and the growth of adventitious roots may prevent dramatic yield loss (Mustroph 2018). There are also examples like in Japan, where sunflower is grown in rotation with rice in the paddy field and where higher soil moisture provoke decrease in growth, yield, oil content and the oleic acid content (Yasumoto et al. 2011).

Physiological mechanisms responsible for the responses of crops to excess soil moisture is still insufficient and future research should be addressed to QTL analyses or genome-wide association (GWA) studies in combination with specific tolerance traits (Mustroph 2018). Such studies should also include sunflower wild relatives as bearing in mind the diversity of habitats they inhabit information about flooding tolerance among them can enhance the chances of finding useful tolerance traits which can be used in breeding process to improve adaptation of cultivated sunflower.

3.2.6 Salinity Tolerance

As one of the major abiotic stresses in plant production that cause negative effects on plant growth and crop yield, salinity affects more than 800 million hectares of land and in total more than 20% of agriculture (Mickelbart et al. 2015; Song and Wang 2015; Ding et al. 2018; Li et al. 2020). In many countries, sunflower is often grown on low-to-medium-saline soils (Hladni 2010; Škorić 2016). Generally, sunflower is known as a moderate salt-tolerant crop but its ability to survive and grow under saline conditions depends on the interaction between salty environment and the ability of the genotype to tolerate those conditions, which also varies depending on growth phase. The first phase in sunflower life cycle, germination, is significantly affected by the salt stress which reduces the ability of plants to uptake water from the soil, resulting in the growth inhibition and yield loss (Li et al. 2020). The results of numerous studies indicated negative effects of increased levels of salinity on other sunflower traits such as leaf area, dry matter, seed number per head, seed yield per plant, seed yield and seed oil content, particularly adversely affecting sensitive genotypes (Katerji et al. 1994; Flagella et al. 2004; Di Caterina et al. 2007; Jabeen and Ahmad 2012). There are different selection criteria proposed by a number of researchers that can be used in salinity studies such as cell survival, seed germination, dry matter accumulation, leaf death or senescence, leaf ion content, leaf necrosis, root growth, and osmotic (Singh 2000; Lexer et al. 2003; Fernandez-Martinez et al. 2009; Seiler 2012; Škorić 2012; Li et al. 2020). One of the latest approaches for efficient and reliable method for screening sunflower genotypes for salinity tolerance is proposed by Li et al. (2020) who suggested that the germination index and the germination vigour index are the two most reliable traits for salt tolerance of sunflower at the germination stage, based on correlation analysis. Authors phenotyped 552 inbred lines of sunflower for different traits at seed germination stage and developed a quantitative evaluation model. The use of sunflower wild relatives can elevate the success in breeding for salt tolerance as several *Helianthus* species are native to salt-affected habitats (Seiler et al. 2017). As a unique species well adapted to saline soils *H. paradoxus* is a great source for salt tolerant genes which can be used to breed more salt-tolerant cultivated sunflower and potentially provide a 25% yield premium in saline soils (Hajjar and Hodgkin 2007; Seiler et al. 2017).

3.2.7 Herbicide Tolerance

Imidazolinones (IMI) and sulfonylureas (SU) tolerant plants with altered *AHAS* genes and enzymes have been widely used since their introduction in the early 1980s, and now they constitute one of the major weed control methods for many crops (Tan et al. 2005; Škorić 2009). Gene discovery and trait development for herbicide tolerance in sunflower is considered to be one of the most important issues when it comes to raising the productivity and the competitive ability of this crop (Sala et al.2012b). IMI and SU herbicides provide excellent broad-spectrum weed control in sunflower, including some of the most problematic weeds. In addition, both families of herbicides allow the possibility to control weeds that are insufficiently controlled by other herbicides traditionally used in sunflower (Sala et al.2012a).

In 1998, USDA-ARS (NDSU) research team transferred IMI resistance into cultivated sunflowers and released public “IMISUN” lines (Miller and Al-Khatib 2000). Malidža et al. (2000), reported having transferred resistance to Imidazolinones from the wild *H. annuus* L. from Kansas into the elite line HA-26 and found that the resistance was controlled by a single partially dominant gene. Sala et al. (2008a, b) identified new source of IMI resistance, CLHA-PLUS, that is controlled by the expression of the partially dominant nuclear allele *Ahasl1-3* that was developed by seed mutagenesis and selection with imazapyr. At the molecular level that CLHA-PLUS is found to be different from *Imr1* and that both of them are allelic variants of the locus *AHASL* (Sala et al. 2008a, b). This new trait confers better stability of the herbicide tolerance in different environmental conditions, permit developing new herbicide formulations providing more flexible and reliable weed control, higher oil content, than previous IMISUN trait (Sala et al.2012a, b; Weston et al. 2012). Sulfonylurea-tolerant sunflowers were developed from wild sunflower populations discovered in the United States collected from the same Kansas area in which IMI resistance was found. The USDA-ARS (NDSU) research group incorporated this genetic resistance into cultivated sunflower and released public lines SURES-1 and SURES-2 in 2001 (Al-Khatib et al. 1999; Miller and Al-Khatib 2004). This trait, controlled by one dominant gene was introduced into elite inbred lines and sulfonylurea tolerant sunflower hybrids (Express Sun technology) were introduced in the

USA and in many countries in Eastern Europe (Fabie and Miller 2002; Sarpe et al. 2007; Jocić et al. 2008, 2011; Mithila and Godar 2013; Meluca et al. 2014; Cvejić et al. 2016).

Although Clearfield and Express Sun technologies are widely used, there are problems in the production such as herbicide residue effects on the following crops, gene escaping to wild species, weed tolerance, as well as tolerant sunflower cultivars response to ALS inhibiting herbicides (Sala et al. 2008a, b, 2012a; Vrbničanin et al. 2008; Božić et al. 2012; Presotto et al. 2012; Jursik et al. 2015).

3.2.8 Nutrient Use Efficiency

Indices of nutrient use efficiency (NUE) in plant production are often the ratios of mass balances between crop yields and the total amount of nutrients in plants in harvest. Term NUE is also being used for nitrogen use efficiency, as a parameter for improving crop sustainability as well as indicator of progress towards worldwide food security, improved nutrition, reduced pollution and sustainable agriculture (Norton and Roberts 2015). Nutrient deficiency and/or nutrient toxicity is not yet recognized as a main target in sunflower breeding programs worldwide (Seiler 2012; Škorić 2012). Using the appropriate screening methods, selecting adequate genetic material, and using available breeding methods the NUE in sunflower can be increased. When it comes to the potential for creation of varieties with superior NUE it is important to note that it depends largely on the genetic variability present for that particular NUE-regulated property along with the development of a methodology for the precise quantification of physiological parameters that reflect an effective NUE (Baligar et al. 2001).

Nutrients have essential importance in plant metabolism and due to disruption of the supply of a particular nutrient, changes in plant metabolism are manifested. Like other crops, sunflower is highly dependent on nutrient availability, which is ultimately expressed through quantitative and qualitative characteristics of sunflower achene. According to Blamey et al. (1987) the most important macro elements for sunflower development are nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca) and magnesium (Mg). Each mineral element has specific function in plant metabolism. Nitrogen (N) is one of the most needed elements for plant growth participates in processes of photosynthesis, respiration, multiplication and cellular differentiation, while phosphorus (P) plays an important role in the maintenance of membrane structures, synthesis of biomolecules and formation of high-energy molecules (Malhotra et al. 2018; Xu et al. 2020). In sunflower production, farmers traditionally apply organic fertilizer plus N and P fertilizers, which are considered as the nutrients that most frequently limit plant growth (Shu-Tian et al. 2018). Besides N and P, other factors such as water availability, environmental conditions and other nutrients as well co-limit plant growth (Weih et al. 2018). Among nutrients, potassium (K) is very important for cell growth and plant development, it has strong mobility in plants and plays an important role in the formation of the yield and quality improvement (Hepler et al. 2001; Oosterhuis et al. 2014; Hu et al. 2016). It has also important role in increasing cell wall thickness and tissue stiffness and thus increasing resistance to some pathogens (Basseto et al. 2007). The application of K in sunflower increases achene yield, 1000-achene weight and kernel rate of both oil and confectionery sunflower (Shu-Tian et al. 2018). Sunflower is also highly sensitive to boron (B) deficiency, as it is an essential microelement which plays an important role in transporting nutrients from the roots to upper parts of the plant, its deficiency is widespread around the world with negative effect on crop production (Ceyhan et al. 2008; Wang et al. 2015). For balanced plant growth and exploitation of seed yield potential, as well as adequate oil and fatty acid content it is necessary to provide optimum amounts of these macro elements during the entire vegetation season as imbalanced mineral supply not only influences sunflower achene yield but also decreases other nutrient efficiencies (Amanullah and Khan 2010; Shu-Tian et al. 2018). Baligar et al. (2001) reported that efficiency of applied fertilizers in sunflower have been estimated to be about or below 50% for N, below 10% for P, and about 40% for K.

3.2.9 Water Use Efficiency

Bearing in mind ongoing climate change one of the main goals of water management will be the implementation of effective water management strategies as a key element for increase in water productivity. As outlined in study of Hatfield and Dold (2019), changing climate with increase in the temperature, variable precipitation and increase in concentration of carbon dioxide (CO_2) will affect water use efficiency (WUE) in plants. Furthermore, these climate changes will also have an impact on increased atmospheric water demand by crops and increased potential for soil water availability due to increased variation in precipitation during the growing season. WUE is defined as the ratio of amount of carbon assimilated as biomass in the plant and the total loss of water due to transpiration (Blum 2005). Unlike other field crops sunflower forms a deep root system allowing it to draw water from the deeper layers of the soil. Because of that water requirements for sunflower are moderate and it can tolerate short-term drought with moderate yield reduction (Garcia-Vila et al. 2012). However, the long-term water deficit, which is increasingly common due to climate change, is limiting evapotranspiration and thorough reduced assimilation of carbon negatively affect the growth of sunflower plants, and consequently seed and oil yields (Demir et al. 2006; Ahmad et al. 2014). Although sunflower has ability to withstand short periods of drought, the lack of water affects all development phases of the sunflower. The most effective strategy for reducing the negative effect of water scarcity in sunflower production is supplementary irrigation, especially in late vegetative period, flowering period and seed formation (Mahmoud and Ahmed 2016; Xiao et al. 2007). Enhancing WUE of plants is probably the most effective and the cheapest strategy to deal with negative effects of water deficit and climate change. This can be achieved through selection and creation of genotypes with changes in morphology and other characteristics that will enhance WUE (Rauf 2008). Mahmoud and Ahmed (2016) evaluated WUE in new sunflower genotypes obtained from selling and induced mutation and results indicated that mutation $M_{2,1}$–63 surpassed other genotypes. Some authors suggest that selection for a higher WUE can be achieved through indirect selection on isotope Δ13C content which is of a great importance in sunflower breeding programs aimed at generating drought tolerant genotypes (Lambrides et al. 2004; Canavar et al. 2014). Applying biotechnological approaches in selection for WUE can lead to a more effective and faster results in breeding process. Water uptake and loss in plants is influenced by developmental phase via environmental factors, there is a complex network of genes that regulates root morphology and architecture, cuticle development, stomatal development, etc. and strongly impact water use efficiency (WUE), and represent the best targets for molecular breeding programs (Ruggiero et al. 2017).

3.3 Sources of Abiotic Stress Tolerance Genes

3.3.1 Wild Relatives as a Source of Abiotic Stress Tolerance

Greater diversity present in wild relatives allows adaptation to environmental changes and challenges and thus preserve economic viability of cultivated sunflower. Genus *Helianthus* is consisted of 39 perennial and 14 annual species that are invaluable source of many useful traits (Seiler et al. 2017). The full exploitation of wild relatives is hampered by the fact that genus *Helianthus* genus contain species of various levels of ploidy. Implementation of specialized techniques such as embryo rescue or tissue culture is required for obtaining interspecies hybrids and transfer of gene of interest. Improvement of cytogenetic studies increased the success and enhanced introduction of genes for many important traits as differences in the ploidy level cause many difficulties such as cross incompatibility, embryo abortion, sterility, reduced fertility, and dormancy in interspecific hybrids. Breeding for resistance is considered to be the most effective and environmentally friendly practise for biotic and abiotic stress control. The use of wild relatives of sunflower in breeding have enabled so far to maintain the resilience and sustainability of the seed and oil yield of cultivated sunflower and thus preserve economic viability. During domestication process, cultivated sunflower lost many of the traits from its wild progenitor, especially in seed characteristics, plant architecture changes, changes in reproductive strategy and life cycle shift (Radanović et al. 2018). Breeding for modern crops should include traits that are lost during domestication process and selection for high yield, primarily meant on adaptations to adverse environments (Palmgren et al. 2015). Sunflower wild relatives represent valuable source for tolerance to different abiotic stresses such as drought and nutrient stress tolerance found in *H. anomalus* (Seiler et al. 2006; Seiler 2007). Drought tolerant species are also *H. argophyllus*, *H. mollis*, *H.*

deserticola, *H. hirsutus*, *H. maximiliani*, *H. tuberosus* etc. as presented in several studies (Baldini and Vannozzi 1999; Škorić 2009; Vassilevska-Ivanova et al. 2014). In the study of Welch and Rieseberg (2002) *H. paradoxus* was found to be five times more salt tolerant than ancestral species *H. annuus* and *H. petiolaris*. High adaptation of *H. paradoxus* to salinity is confirmed in several other studies (Chandler and Jan 1984; Miller 1995; Edelist et al. 2009). Because of high adaptation to desert and sandy areas *H. anomalus* Blake and *H. deserticola* Heiser are good candidates for breeding for heat stress tolerance (Seiler 2012). Benefits of using wild relatives is also the opportunity to study the physiological processes that are involved in the survival mechanisms which may be useful for improvement of cultivated crops grown under abiotic stress. This is outlined in study of Bowsher et al. (2016) who tested the expectation that a desert-dwelling sunflower species, *Helianthus niveus* ssp. *tephrodes* would exhibit root and leaf traits consistent with greater ability to avoid drought than cultivated sunflower *H. annuus* in a usual garden environment. The results from their study reveal that leaf pubescence from *Helianthus niveus* ssp. *tephrodes* may give improvement in breeding for drought-prone, high radiation environments. Evolutionary changes in sunflower cultivation in the last two decades has been achieved through the introduction of aceto-hydroxy acid synthase (AHAS) genes for herbicide tolerance from wild population of *Helianthus annuus* L (Sala et al.2012c).

3.3.2 Local Populations, Open Pollinated Varieties

The importance of local populations and open pollinated varieties as a gene pool for crop improvement is reflected in the fact that they are characterized with many valuable genes, especially those addressing higher adaptability to specific environmental conditions and resistance to certain diseases. Sunflower breeding as an industrial plant started in Russia, where farmers initiate selection of individual plants based on phenotype characteristics. Thus, Russian farmers were among the first "sunflower breeders" in modern history as they were choosing individual plants based on characteristics like head size, seed size and stay-green characteristic. Using the method "mass selection", a number of local varieties were created among which well-known were Zelenka, Chernyanka, Puzanok and Fuksinka (Pustovoit 1967). Using phenotypic selection Russian farmers created a large number of local cultivars of which some had improved agronomic traits and resistance to important constrains of sunflower production (Seiler and Jan 2010). Škorić (2016) reported that use of sunflower landraces and varieties made some practical results in breeding for drought tolerance. Open pollinated varieties created in Russia have been used as a base for breeding programs around the world and with the introduction of hybrids in production served as the source for development of a large number of lines. Even today in some breeding Institutes in Russia, in a smaller part of assortment, offer varieties tolerant to drought and other stresses like R-453 (Rodnik), Buzuluk, Umnik (https://en.vniimk.ru/).

3.3.3 Inbred Lines Gene Pool for Abiotic Stress Tolerance

The great importance of genotypes created in different breeding canters is reflected in diversity of environments of their origin and considering that sunflower breeding canters exist all over the world, it is a really wide range of different environments. These genetic stocks have certain limitations for their use, agreements are generally required. They are made up of different types of synthetic populations and inbred lines created over many years of breeding and using different tools for broadening the variability such as induced mutation, interspecific hybridization and new biotechnology methods. Certainly, the most famous sunflower collection of public sunflower inbred lines is USDA-ARS. Sunflower germplasm from USDA-ARS was and still is distributed around the globe and served as the base breeding material for creation of numerous B, A and Rf lines. Usually, germplasm from this centre is used in recurrent selection for introduction of many valuable genes (Seiler and Jan 2010; Terzić et al. 2020). Using interspecific hybridization between cultivated *H. annuus* and *H. paradoxus* Miller and Seiler (2003) released two salt-tolerant oilseed parental lines, HA 429 and HA 430. Sunflower inbred lines having stay-green traits such as HA-48, HA-22, CMS-1–50, PH-BC-2–91, PR-ST-3, RHA-SES and RHA-583 are suitable for the development of drought-tolerant lines and hybrids (Škorić 2016).

Other important sunflower breeding canters which are necessary to mention because of their considerable contribution to sunflower genetic resources especially in breeding for resistance and tolerance to diseases and herbicides are Institute of Field and Vegetable Crops from Novi Sad, Dobroudja Agricultural Institute from General Toshevo, National Institute for Agricultural Research from Montpellier, Instituto Nacional de Tecnología Agropecuaria from Pergamino, Institudo de Agricultura Sostenible from Cordoba, Institute of Oilseed Crops from Zaporozhie and well known Vavilov All-Russian Institute of Plant Genetic Resources from Saint Peterburg.

3.3.4 Artificially Induced/Incorporated Traits/Genes

Generating new genetic variability, mutations are the primary source for alterations of traits in living organisms. There is no difference in variability caused by spontaneous and induced mutations, except of higher rate of induced mutations. In mutation breeding, different mutagenic agents are used in order to generate new genetic variability with desirable traits, based on random genetic variations. Mutagens have potential to induce hereditary alterations in plant genome and thereby enhance the frequency to obtain preferred individuals. Mutagenic agents have been used in breeding by many authors but have generally been restricted to obtaining dominant traits while recessive ones have largely been lost during selection (Barkley and Wang 2008). Climate drift has increased the need for mutation breeding in order to develop germplasm with increased resistance to abiotic stress. This includes application of induced mutations in order to improve plant architecture, shift in life cycle and resistance/tolerance to stresses. It is possible to enrich the genetic variability of cultivated sunflower using mutations (Cvejić et al. 2014). Many useful traits in sunflower have been already developed with induced mutations by applying physical and chemical mutagenic agents such as shorter vegetation, larger head and increased 1000 seed weight, decreased plant height, resistance to rust and broomrape, altered oil content and quality and tolerance to herbicides (Savin and Stepanenko 1968; Cvetkova 1970; Soldatov 1976; Lofgren and Ramaraje Urs 1982; Schuster and Kubler 1983; LeClercq 1985; Osorio et al. 1995; Velasco et al. 1999; Sala et al. 2008a, b; Encheva et al. 2008; Encheva and Shindrova 2011 Mykhailenko et al. 2019). In mutation breeding it is very important to use an effective concentration of a mutagen as the Mykhailiuk frequency and range of mutations is also determined by adequate application (Mykhailenko et al. 2019).

3.4 Genetic Diversity Analysis

Compromises between both stress resistance and plant performance have been well documented in natural and domesticated germplasm alike (Kempel et al. 2011; Koziol et al. 2012; Lind et al. 2013). They also represent the primary explanation for the decline in resistance that has been reported in numerous crops compared to their wild progenitors (Koziol et al. 2012). During the process of adaptive divergence, genomic regions under selection can display strong differentiation. While the ongoing gene flow between populations homogenizes other regions, thus generating heterogeneous patterns of genomic divergence (Wu 2001; Nosil et al. 2009).

Future climate change and adaptation of cultivated sunflower to the new abiotic environment require the use of knowledge of where wild germplasm are locally adapted today (Mehrabi et al. 2019). It was established that sunflower often thrives outside its canters of origin and wild diversity. Domesticated sunflower is cultivated across wide areas of temperature and precipitation gradients that, on a global level, seem to outstrip the narrow spread climatic space of wild relatives of sunflower crop in their native range in Northern America (Khoury et al. 2016). Domestication and sunflower breeding create population bottlenecks and eroded genetic diversity (Tang and Knapp 2003; Harter et al. 2004; Liu and Burke 2006). However, a great diversity of sunflowers and migration has apparently partially counteracted the effects of domestication and other diversity-reducing processes in modern oilseed sunflower inbred lines (Cheres and Kanapp 1998).

Land races and OP varieties have numerous genetic variations. They are also well-adapted to the local soil types and climatic conditions, in addition to other environmental factors. They are the source of many desirable genes, most importantly the genes addressing higher adaptability to environmental conditions (Kaya et al. 2015). Sunflower landraces contribute with diversity to the improvement of high yielding oilseed and confectionary sunflower hybrids (Tan and Kaya 2019). This genetic diversity and its characterization are particularly important for hybrid sunflower breeding. This is due to the fact that parental lines with the diverse origin have a higher potential heterosis, compared to hybrids created from closely related parents (Kaya 2016).

3.4.1 Phenotype-Based Diversity Analysis

Cultivated sunflower in most casses has limited genetic variability. This is especially true for major agronomic traits (Zambelli and León 2015). Therefore the creation of a new sunflower ideotype demands broadening of the sunflower genetic diversity and it asks for an increased use of wild *Helianthus* species in breeding programs (Škorić 2012). Phenotypic diversity of the tested material is determined using distinctness, uniformity, and stability (DUS) test guidelines (UPOV 2000).

There has been substantial progress when it comes to collecting and preserving wild species, understanding the origin, domestication increasing the genetic diversity and organization of the genetic diversity characterization and screening methods for abiotic stresses in sunflower so far, only a small portion of the available diversity has been exploited (Seiler 2012; Seiler et al. 2017). Modern crops should be developed with properties once possessed by their CWR (crop wild relatives) in order to tolerate adverse environmental conditions that were inadvertently lost during selecting for high yield (Palmgren et al. 2015). This is why, knowledge of distinct habitats and adaptations of wild species can be helpful in identifying potential sources of tolerance genes in ecotypes which are able to survive in areas with abiotic challenges (Seiler et al. 2017). Sunflower CWR contain significant variability when it comes to tolerance to abiotic stresses which include drought, salinity, heat, flooding, low nutrient, and heavy metal tolerance (Ortiz 2015).

For the germplasm collections to be used more efficiently and effectively, it is important to characterize the diversity of the germplasm (Anđelković et al. 2020; Terzić et al. 2020). The most common technique used in the estimation of the genetic diversity of the crops is based on both pedigree records and phenotypic trait observation. Traditionally, morphological traits are used for individual evaluations of both the uniformity and stability of the genotype. by Coque et al. (2008) and Mandel et al. (2011) have exhaustively addressed the genetic diversity and population structure of North American and European resources. Terzić et al. (2019) confirmed the UGA-SAM1 as an important resource when it comes to sunflower research. The highest phenotypic diversity was found in the less developed group, followed by non-oil genotypes that have been developed from less intensive selection compared with oil lines and more so when compared with the RHA-O group, which had demonstrated the lowest diversity. What was interesting is that the RHA-O group had the highest diversity for specific traits including days to maturity, which was the result of optimization of HA lines for pollination and less intensive exclusion of early genotypes due to lower yields. Hladni et al. (2017) used the Shannon (H) diversity index in order to evaluate the variability of 68 confectionary sunflower genotypes based on 32 morphological descriptors. The high diversity index value (0.7) signifies that the material evaluated is representative as a confectionary sunflower germplasm collection. HOMALS analysis, demonstrates the value of descriptor selection for germplasm evaluation. Based on the HOMALS analysis, the most instructive traits were coloration of stigma DFIA, seed colour of stripes SCS and seed main colour SMC which had the highest variability. It also had the highest discriminative power among genotypes. Isolated genotypes that may not be represented by yield or seed-specific traits can be useful sources of traits used in breeding. If the confirmed diversity of the studied material is taken into consideration, it is safe to assume that it possesses a good potential when it comes to long-term use for adaptability of confectionery sunflower abiotic stresses tolerance.

The efficiency of breeding programs can be improved by adequate selection of traits used in germplasm evaluation. While the loss of variability can be lowered if diversity focused traits were used. This includes leaf, seed, and certain flower traits, instead of only focusing on yield and quality (Terzić et al. 2019). Finding the most productive sunflower hybrid for specific environmental conditions is possible via testing in different environments, if we take into consideration that the genotype is fixed while the environment shows variation through years and locations. Reliable estimation of the extent to which genotype by environment interaction (GEI) affects a trait of interest is an important determinant for the evaluation and selection of genotypes, especially for target regions. For obtaining reliable information regarding the GEI effect, it is necessary to organize a multi-environment trial (Jocković et al. 2019).

3.4.2 Genotype-Based Diversity Analysis Based on Molecular Marker Studies

Implementation of the molecular biology techniques in plant breeding is of exceptional value. It is more profitable for plant breeder as it facilitates breeding strategies and ensures exact and quick results (Duca 2008). Molecular markers provide an effective means for characterizing genetic variability and establishing phylogenetic relationships among cultivated and wild *Helianthus* species. Markers linked with both qualitative and quantitative traits and genes should make marker-assisted selection (MAS) easier. Eventually leading to cloning and manipulation of desirable genes. When it comes to understanding the origin, domestication, and organization of the genetic diversity, characterization, and screening methods for abiotic stresses significant advances have been made (Seiler and Jan 2010).

For sunflower, simple sequence repeats (SSR) markers have been developed. In addition, they have been used in one study to characterize the genetic diversity among 16 elite inbred lines, and in another study among 19 elite inbred lines and 28 domestic and wild germplasm accessions, this also included Native American landraces (Paniego et al. 2002; Tang and Knapp 2003). “Target region amplification polymorphism” (TRAP), a new marker technique, has been developed by Tang and Hu and Vick (2003). It uses bioinformatics tools and expresses sequence tag (EST) database information to generate polymorphic markers around targeted gene sequences. To investigate the loss of genetic diversity associated with artificial selection, 266 accessions of *H. annuus* were analysed, including 239 cultivated accessions used for GWA analyses, as well as 27 wild accessions spanning the geographic range of *H. annuus* in North America. (Hubner et al. 2019). The nucleotide diversity parameter (p) (Nei 1987) was calculated on a per-site basis. Determine the nucleotide diversity of each gene, the average p value was calculated across all single nucleotide polymorphisms (SNPs) within the same gene. For the wild and cultivated accessions these calculations were performed separately. The reduction in genetic diversity associated with artificial selection was estimated by the ratio of diversity values in wild sunflowers to those in cultivars (p wild/p cultivated) (Gao et al. 2019). The largest and most comprehensive analysis of genetic diversity, population structure and linkage disequilibrium for cultivated sunflower conducted to date INTA, INRA and USDA-UBC. The genotyping strategy that was used combined proprietary ddRADseq with public WGS data to with the goal of obtaining an integrative SNP-matrix. It also included individuals from different breeding programs. In this regard, gene banks and CWR collections hold a substantial amount of genetic diversity for many agronomically important traits that are available to be exploited to expand the breeding genetic base. As well as to deal with the changing environmental challenges for the crop (Filipi et al. 2020).

In this day and age, the increased availability of SNP markers, along with their more rapid and highly automated genotyping technologies, have been the driving force that has inspired their use in diversity studies and the evaluation of population structure (Mandel et al. 2013; Cadic et al. 2013). Ecological selection can be both quite complex and environmentally dependent. In addition, it can act in different genomic regions at different life history stages. Ćalić et al. (2016) have been able to identify a set of nearly 500 K high-quality SNPs from whole-genome shotgun re-sequencing data. They werea also able to use this data, along with a custom GWAS pipeline, to investigate the genetic basis of abiotic stress responses in sunflower. Goebl et al. (2020) have studied how populations adapt to new habitats. From how small populations persist despite ongoing gene flow to the conservation of diversity. What they discovered is that the maintenance of divergent adaptation in this system is mediated via habitat and life stage-specific selection how that alters allele frequencies. The possibility to observe allele frequency shifts during early life stages at specific loci is particularly important for selection (Huang et al. 2020; Todesco et al. 2020). Filipi et al. (2015) stated in their first report that comparing the performance of SSR and SNP markers for population genetics analysis in cultivated sunflower showed that both the SSR and SNP panels used are equally appropriate for estimating genetic diversity and population structure in sunflower association mapping population. The levels of diversity and population structure of these inbred lines provide us with generated knowledge which is an important contribution to sunflower breeding and conservation. It also serves to complete the worldwide diversity map of cultivated sunflower.

The primary gene pool that occurs in extreme environments indicates that utilization of wild *H. annuus* for the breeding of abiotic stress tolerance is likely to produce quick gains with minimal effort. Especially when using a combination of gap analysis, environmental niche modelling, and phylogenetic approaches. The combination of techniques demonstrates the potential for publicly available ecogeographic and phylogenetic data that can further facilitate identification of potential sources of abiotic stress traits in plant breeding programs (Kantar et al. 2015). Hladni et al. (2018a) reported a similar result where the combination of the PCA of morphological data, PCoA of molecular marker data and GD between parental lines is fast and affordable. But more importantly, at the same time it provides crucial information for parental choice. Knowledge of the complex response of the plant to the abiotic stress requires avoidance of the single gene analysis and advancing in the genome information of the sunflower (Mianlengeh et al. 2018). Studies of the *Helianthus* species will be highly relevant for research concerning characters for which they are the only source and for understanding evolution and adaptation of the Helianthus genome (Terzić et al. 2020). It is important to note that there has not yet been a comprehensive analysis of the genetic diversity and allelic variants currently being used across international breeding programs. Performing these types of studies is a critical step towards a better understanding of the genetic base of current sunflower breeding worldwide (Seiler et al. 2017). It is necessary to apply an integrated multidisciplinary approach based on plant genetics and genomics, physiology, and modelling (Sala et al.2012a, b, c). The combined use of pheno- and genotyping can also be considered the future of UPOV DUS test. In this usage genetic distances translated to UPOV characteristics are proposed as an addition to existing phenotype traits (Terzić et al. 2019).

3.5 Classical Genetics and Traditional Breeding for Abiotic Stress Tolerance

The cultivated sunflower has a long history beginning with the Native American Indians' domestication as early as 3000 BC developing a tall single-headed plant type for oil extraction, dye, and medicinal use. During the 1500 s, sunflower was primarily used as forage for livestock and poultry in Europe as well as in the Americans after it was introduction to the US in the 1920s to 1940s. The first revolutionary change in sunflower was its establishment as an oilseed crop in the 1950s with an intensive breeding effort to produce high oilseed sunflower. Soon after, the second revolution was the conversion of sunflower to a hybrid crop utilizing hybrid vigour via the use of cytoplasmic male-sterility and fertility restoration gene quickly establishing sunflower as one of the major oilseeds crops worldwide (Seiler et al. 2017).

During the past 70 years, the modern-day high oilseed sunflower has benefitted from the advancement of breeding for all the aspects of agronomic characteristics, diseases resistance, and quality, but the pace of improvement has plateaued due to its narrow genetic base. The value of the large genetic resources of the wild *Helianthus* species has been recognized with their utilization through interspecific gene transfer contributing new genes for agronomic and quality characteristics, as well as biotic and abiotic environmental stresses (Terzić et al. 2020). Due to the narrow genetic base of cultivated sunflower and the utilization of the wild *Helianthus* species, significant progress has occurred in recent years increasing resistance to diseases, insects, broomrape, oil and oil quality, and abiotic stress including salt, herbicide, and drought tolerance. The wild *Helianthus* species are widely distributed in North America, from northern Mexico to southern Canada, from the Pacific to the Atlantic Ocean, and in high mountains to 2600 m, in alkaline, salty, dry and wet soils, each with specific adaptation features enduring their natural and often extreme and hostile habitats (Rogers et al. 1982). Their survival mechanisms to the extreme abiotic stress should be among the high priorities of any sunflower breeder's future planning for improving stress tolerance in cultivated sunflower. Previous reviews of sunflower response to abiotic stresses (Škorić 2009, 2016), sunflower and climate changes (Debaeke et al. 2017; Miladinović et al. 2019), and wild and cultivated sunflower genetic resources (Terzić et al. 2020) have paved the way for this discussion.

3.5.1 Root Characters

In the past decades, breeders were mostly occupied with improvements of above-ground parts of sunflower plant neglecting one of perhaps the most important parts of the plant for its ability to adapt to different environmental conditions. Because of that, it is necessary to focus future breeding efforts on improvement root traits, as well, in order to minimize the effect of stressful conditions on plant development. These efforts could be facilitated by novel phenotyping platforms for non-invasive root analysis, that enable efficient characterization of root architecture and investigation of the developmental dynamics and root growth (Radanović et al. 2018).

Kaya (2016) stated that in sunflower, drought tolerant genotypes should be attributed with powerful roots in order to increase water uptake from the soil. Cultivated sunflower has the potential to trap soil moisture reserves that are inaccessible to many other crops and root traits. This includes root length and diameter, root length density, root volume, fresh and dry root weight. Along with total dry matter are significant indicator of sunflower root drought tolerance (Song et al. 1999; Rauf 2008; Comas et al. 2013). However, there is a lack of useful data regarding both the morphological and the physiological root parameters that can be useful in sunflower breeding. This is due to the fact that it requires the knowledge about root traits and their effect on sunflower productivity while it is known that depth-efficient roots for more water uptake are one of indicators of physiological drought tolerance (Comas et al. 2013; Kaya 2016). Ongoing climate drift in recent years motivated scientists for thorough evaluation of plant performance under stress conditions including detailed physiological studies as well as genomic analyses with special attention that has been given to evaluate relationship between root-related traits and performance under water limitation (Mitchell et al. 2013; Mickelbart et al. 2015; Kusmec et al. 2017; Scoffoni et al. 2017a, b; Li et al. 2018). Results from several studies gave clearer picture about transformations in root morphology under water deficit and it has been noticed that plants often increase the distribution of root biomass and change root morphology under water restriction (Pace et al. 1999; Uga et al. 2011; Tardieu 2012; Comas et al. 2013). Rauf et al. (2009) evaluated sunflower root characteristics under different water regimes and results indicated that drought had repressive effect on root weight and shoot length while elevate effect on root length and root-to-shoot ratio.

Biotechnological tools increased the progress in identification of the genetic basis of root traits variations in sunflower. Quantitative trait loci (QTL) associated with increased root foraging, root length, root biomass, lateral root length, and root angle have been identified (MacMillan et al. 2006; Courtois et al. 2009; Ruta et al. 2010; Uga et al. 2011, 2013; Mace et al. 2012; Christopher et al. 2013; El-Soda et al. 2014; Gao and Lynch 2016). Using genome wide association (GWA) study Masalia et al. (2018) measured several root and growth traits in sunflower in well-watered and water limited environments and identified 13 genomic regions that were associated with the traits of interest across the two environments. The authors found that water limitation reduced seedling size and produced a shift toward deeper rooting and also discovered evidence of pleiotropy across multiple traits, as well as numerous environmentally independent genetic effects. They also suggested that the majority of alleles associated with these traits have consistent effects across environments.

3.5.2 Heat and Cold Tolerance

High heat during flowering and seed-filling stages negatively impacts fertilization and can drastically reduce seed yield. Temperature above 31 °C at anthesis significantly reduces yield because of its negative effects on pollen production and fertilization (Chimenti and Hall 2001). Chimenti et al. (2001) also indicated that the embryo growth continued to decrease with the increase of temperature from 25 to 40 °C. Unfortunately, extremely limited work targeting the genetics and breeding of heat tolerance has been conducted. However, the use of wild *H. argophyllus* (Tavoljansky et al. 2004; Warburton et al. 2017) was suggested for heat tolerance utilizing its silvery hairy leaves to reflects sun lights to reduce the leaf temperature and thus reduce transpiration. Seiler (2012) also suggested to use wild *Helianthus* species *H. anomalus*, *H. deserticola*, *H. nuttallii* Torrey and Gray, and *H. petiolaris* for heat tolerance.

Cold tolerance in sunflower would safeguard the crop from early planting problems related to seed germination, and seedling survival, and would be favourable for stable flowering and seed filling for plants growing in relatively colder environment. Wild *Helianthus* species growing in the far northern latitudes of their distribution and at high mountain elevations are expected to possess genes tolerant to the extreme cold that should be considered for cold tolerance breeding (Škorić 2009). Candidate species could include *H. petiolaris*, *H nuttallii*, *H. arizonensis*, *H. ciliaris*, *H. cusickii, H. pumilus*, *H. maximiliani* Schrader, and *H. pauciflorus* (*rigidus*) (Cass.) Desf. Tetreault et al. (2016) compare the cold acclimation capacity and freezing tolerance of high cold tolerance of wild *H. maximiliani* from Manitoba, Canada to this species from Texas and Kansas. Similarly, only selected northern *H. tuberosus* L. and *H. maximiliani* selection locally adapted to survive the harsh winters in Fargo, ND, USA survived, while the others less adapted ones were quickly eliminated from a perennial test plot (Seiler, personal communication). Of course, the mechanization of cold tolerance of candidate accession(s) should always be examined before any attempt to transfer genes begins.

The results of obtaining cold tolerant genotypes among chemically mutated progenies (Kalaydzhyan et al. 2007, 2009) cited by Škorić (2016) are exceptionally encouraging. The selected progenies planted in late fall/early winter survived the harsh winter and low temperature to −20 °C in the field. These should be an excellent germplasm source for all the sunflower breeders looking for high cold tolerance.

3.5.3 Drought Tolerance

Drought is the most important abiotic stress negatively affecting the sunflower crop globally. Unfavorable seasonal variations of available precipitation continue to threaten its cultivation in drought-prone marginal areas. Sunflower genotypes having the ability to survive water deficits with a deep rooting system and the use of water conservation mechanisms that can resume normal growth afterwards are in high demand, especially when growing in less desirable marginal lands.

According to Škorić (2016), drought tolerance characteristics appeared to be related to deep rooting, more efficient water uptake, high osmotic pressure tolerance, low transpiration ratio, and the ability to recover after wilting under heat stress. Parameters useful for drought tolerance selection have been evaluated, including high osmotic adjustment (Chimenti et al. 2004), high self-fertility (Andre 2004), hypocotyl and leaf growth (Sato et al. 2012), root/shoot ratio in early vegetative growing stage (Petcu et al. 2008), and stay-green trait (Škorić 1992). Recently, Umar and Siddiqui (2018) used chlorophyll fluorescence and stomatal conductance, photosynthetic pigments, leaf water status, osmotic potential, hydrogen peroxide (H_2O_2) content, proline content, and enzyme analyses to distinguish susceptible and tolerant genotypes. Due to the complexity of the drought tolerance manifestation and the large number of morphological and physiological characteristics involved, the selection of parameters used in individual genetic and breeding studies often relies on the contrasting characteristics of the parents involved. A "stay-green" trait has been specifically mentioned by Škorić (2016) for its relatedness to drought tolerance, earlier confirmed by Vrânceanu (2000) as an effective criterion for selection of drought tolerance in sunflower. Drought tolerance line with stay-green characteristic have been shown to have increased RuBisCo proteins, therefore higher photosynthesis, and higher quantum yield of photosynthesis in the leaves, supporting the use of stay-green trait for drought tolerance selection.

The wild annual *H. argophyllus* Torrey & Gray found in sandy beaches of Texas and Florida, with an annual rainfall of 50–100 cm, has been extensively used for sunflower drought tolerance. The drought tolerance mechanisms of *H. argophyllus* were compared with four susceptible cultivated lines in the greenhouse with varying irrigation regimes (Baldini et al. 1993). Under drought conditions, *H. argophyllus* was shown to have higher photosynthetic rates, higher transpiration efficiency, and less leaf area reduction. The greater water content of leaves helped maintain greater photosynthetic activity, which led to a greater dehydration avoidance capability due to its well-developed root system enabling improved water uptake. Similar field experiments conducted by Martin et al. (1992) also indicated that *H. argophyllus* had a lower transpiration, a more efficient stomatal control and osmotic adjustment, and the benefits of a denser root system. Divergent selection based on physiological parameters for drought tolerance on F_1, F_2 and F_3 progenies of a cross between a cultivated line and *H. argophyllus* has been proven effective (Baldini and Vannozzi 1998,1999), with a high level of drought tolerance and yield potential combined in improved sunflower cultivars. This efficiency is likely to rely on the ability of breeder's selection of adequate parameters with respect to individual cross combinations.

Even though the cultivated sunflower is highly susceptible to drought, the natural habitats of its wild relatives often grow in exceedingly dry, sandy, or rocky soils with annual precipitation ranging from <12 to 65 cm (Rogers et al. 1982). These species should be the considered and evaluated in the future search of drought tolerance. The species worth looking at are as follows with the annual precipitation in cm in parenthesis: *H. anomalus* Blake (25–50); *H. arizonensis* R. Jackson (25–50); *H. cusickii* A. Gray (25–60); *H. deserticola* Heiser (12–25); *H. exilis* Gray (50); *H. gracilentus* A. Gray (25–50); *H. laciniatus* A. Gray (25–60); *H. neglectus* Heiser (25–50); *H. niveus* (Benth.) Brandegee ssp. *canescens* Heiser (12–50); *H. niveus* (Benth.) Brandegee ssp. *niveus* (Benth.) Brandegee (12); *H. niveus* (Benth.) Brandegee ssp. *tephrodes* (Gray) Heiser (<12); *H. praecox* Engleman & Gray ssp. *hirtus* Heiser (50); and *H. pumilus* Nutt. (25–60).

3.5.4 Salinity Tolerance

Salinity is considered the second most important abiotic stress to crops after drought, defined as mineral deficiency or excess amount of soluble salts surrounding the root zone (Singh 2004b). As sunflower expands into marginal lands in many countries, tolerance to salinity has become essential and among the top priorities of breeding programs. Despite its importance, unfortunately, only limited work on sunflower salt tolerance has been reported thus far. Chandler and Jan (1984) evaluated wild *H. paradoxus* Heiser, *H. debilis* Nutt. And *H. annuus* accessions known to grow in salty areas using hydroponic tanks with varying NaCl concentrations. In the experiment, cultivated sunflower line HA 89 and *H. debilis* wilted at the concentration of 240–400 mM, while the wild *H. annuus* and *H. paradoxus* survived at 800–1300 mM. It was also shown that the salt tolerance of the *H. paradoxus* was controlled by dominant genes since the hybrids between *H. paradoxus* and cultivated sunflower were as resistant as *H. paradoxus*. Following the initial discovery of the *H. paradoxus* resistance, resistance was successfully transferred into cultivated lines and germplasms released (Miller 1995; Miller and Seiler, 2003). Miller (1995) crossed susceptible cultivated line HA 821 with three salt tolerant *H. paradoxus* accessions, and grew F_1, F_2 and testcross BC_1F_1 progenies in pots in the greenhouse and irrigated with varying concentrations of NaCl. His results suggested one major gene Sa_1 controlled seedling tolerance, and a recessive modifier gene also seemed to affect seedling tolerance.

Since *H. paradoxus* is mostly sub-irrigated in low places with water having 12,000–14,000 ppm NaCl concentration, its exceptional salt resistance was expected (Rogers et al. 1982). Studies of *H. paradoxus* in alkaline soil in the field or in the greenhouse with varying concentrations of soil minerals in irrigation water will further clarify its usefulness helping to sustain sunflower production in poor marginal lands. Meanwhile, in order to further enrich the salt tolerance of sunflower, wild *Helianthus* collections from areas of known alkaline soil should be the first choice for evaluation. This group includes *H. ciliaris* DC., *H. salicifolius* Dietr, and *H. laciniatus*. In addition, species from dry and rocky soils should also be considered if resources allow. This group includes species as *H. laevigatus* Torrey and Gray, *H. smithii* Heiser, *H. eggertii* Small, *H. californicus*DC., and *H. gracilentus*.

3.5.5 Herbicide Tolerance

For cultivated sunflower, effective herbicide use will not only help control weeds, but also offer the added benefit of controlling broomrape (*Orobanche cumana* Wallr.), a parasitic weed causing severe economic crop losses if not controlled. Broomrape resistance breeding has been under extreme pressure trying to keep pace with the rapid race shifts in the last 20 years in major sunflower production regions of the world. Herbicide resistant sunflower hybrids could offer an alternative, as well as an additional option to the use of race-specific broomrape resistant hybrids. The advantage of using an herbicide that it is non-race-specific can extend its effectiveness for a much longer period of time than the use of race-specific-resistance genes until broomrape emerges with genes resistant to the particular herbicide.

Sunflower breeders have successfully identified herbicide resistance genes from wild *H. annuus* L. and through chemical induced mutation with resistance to the widely used ALS-inhibiting herbicides, including the imidazolinone and sulfonylurea herbicide. Their mode of action and resistance have been evaluated and incorporated into cultivated lines. The first source of imidazolinone and sulfonylurea herbicide resistance was found from the surviving wild *H. annuus* plants in a soybean field after seven consecutive years of imazethapyr treatment for controlling weeds. This source was quickly transferred into cultivated background by Miller and Al-khatib (2000), Alonso et al. (1998), and Al-Khatib and Miller (2000). The imazethapyr used by Alonso et al. (1998) also provided 100% control of broomrape.

The resistance to imidazolinone herbicides involves two genes, a semidominant *Imr*1 and a modifier gene *Imr*2, with complete resistance achieved only when a line or a hybrid is homozygous with both genes, *Imr*1 *Imr*2 (Miller 1995). A different source of IMI resistance was obtained through EMS treatment was reported by Sala et al. (2008a, b), and the gene CLHA-PLUS was shown to have a higher degree of IMI resistance than that of the wild *H. annuus* source. The BASF's IMI-resistant hybrids, CLEARFIELD®, has since gained wide acceptance by the sunflower breeding communities especially in areas of heavy broomrape infestations. Similarly, the SU-group of herbicide resistance was also identified in the same wild *H. annuus* population possessing IMI resistance, as well as from mutagenesis. Because of the partial dominance of the resistance genes, more effort is needed to incorporate them into the hybrids slowing their acceptance compared to the more popular IMI chemistries of CLEARFIELD®.

The herbicide resistance in sunflower presents a good example of utilizing induced mutation as well as the wild *Helianthus* species. In addition, an abundance of herbicide tolerance genes in wild *Helianthus* species has been demonstrated. Olson et al. (2004) found imazamox and tribenuron tolerance genes after evaluating 46 populations of wild *H. annuus* and *H. petiolaris* Nutt. Miller and Seiler (2005) also reported the discovery of tribenuron resistance genes in collections of wild *H. annuus* populations from Canada. It is likely that wild *Helianthus* species will continue to provide future herbicide tolerance genes when new herbicides are developed for the sunflower crop. However, the heavy use of herbicide tolerant sunflower on large scale production is also likely to accelerate the flow of tolerance genes into nearby cross-compatible wild annual *Helianthus* species, with the herbicide application acting to select for surviving tolerant individuals and quickly increasing tolerance gene frequency in those population making their control impossible. This reverse gene flow should be kept to a minimum and closely monitored.

3.5.6 Traditional Breeding Methods

Tolerance to abiotic stress is usually controlled and affected by multiple, interacting mechanisms and because of the complexity and insufficient research it is especially difficult to breed for. With the use of conventional breeding plant genome is manipulated using conservative breeding tools within natural limitations (Acquaah 2015). Conventional breeding has been used in sunflower for successful development of cultivars (varieties and hybrids) for varieties of eco-environments, adapted to different types of abiotic stress. Selection of the appropriate breeding method firstly depends on genetic background of inherited trait, whether is qualitative or quantitative. Most of the abiotic stress related traits in sunflower are quantitative and controlled by several genes (Table 3.2).

Table 3.2 Abiotic stress tolerance related genes and gene actions in sunflower

Abiotic stress	Gene(s)	Gene action	References
Root characters	Multiple genes	Epistasis	de Dorlodot et al. (2007), Cooper et al. (2009)
	Multiple genes	Additive gene action	Comas et al. (2013)
Heat tolerance	High molecular weight HSPs; Heat stress transcription factor HSFA		Kumar et al. (2003)
Cold tolerance	*COR* genes	Low-temperature signalling	Hewezi et al. (2006)
Drought tolerance	Different cDNAs		Cellier et al. (1996)
	QTLs on Chs 7 and 16	Plant water status traits	Kiani et al. (2007a)
	QTLs on Ch 16	Water status traits	Ebrahimi et al. (2008)
	QTLs on Ch 5	Osmotic adjustment	Kiani et al. (2007b)
	Multiple genes	Additive effect Stay-green	Cukadar-Olmedo and Miller (1997)
Salinity tolerance	*HT089*, *HT175*, *HT185*, *HT215*, *HT 216* and *HT227*	Regulation of uptake of mineral ions	Lexer et al. (2003)
	EST	Codes for the Ca-dependent protein kinase	Lai et al. (2005)
Herbicide tolerance—Imidazolinone herbicides		Single partially dominant gene	Malidža et al. (2000)
	Imr1 and *Imr2*	Semidominant type of gene action	Bruniard and Miller (2001)
	Ahasl1-1	Partial dominance	Sala et al. (2012a, b, c)
Herbicide tolerance—Sures herbicides	*Ahasl1-2*	Partial dominance	Kolkman et al. (2004)
		Dominant type of gene action	Cvejić et al. (2016)

Traditional breeding methods, such as recurrent selection, are widely used in sunflower for the development of improved genotypes resistant and tolerant to different limiting factors in production. Backcross method was commonly used strategy for introducing disease resistance into high yielding inbred lines of sunflower (Jocić et al. 2015). The conventional breeding procedures use hybridization and phenotype-based selection that are later followed by selection of promising breeding lines through yield evaluation trials (Janila et al. 2016). Main goal in sunflower breeding program is development of hybrids with high oil productivity, via seed yield. In order to achieve high productivity sunflower hybrids should be adaptable to different environments and resistant/tolerant to many biotic and abiotic limitations. Bearing this in mind, sunflower development as an industrial crop has undergone three different breeding methods known as mass selection, individual selection and development of hybrids (Jocić et al. 2015). Mass selection was firstly used in sunflower breeding for development of number of varieties with improved seed yield, oil content and resistant for different pests (Pustovoit 1967). Individual selection or "modified recurrent selection" called seed reserve Pustovoit used for improving the oil content in the first half of twentieth century and it is still found to be an effective breeding method in selection for high oil content. After the discovery of cytoplasmic male sterility (CMS) in interspecific hybrid *H. annuus* x *H. petiolaris* by French scientist Leclercq (1969) and use of fertility restorer genes (Kinman 1970) it made revolutionary changes in sunflower production as it was possible to grow hybrids. Exploitation of heterotic potential in hybrids includes the creation of inbred lines and testing the general and specific combining abilities in order to develop superior hybrids. By crossing of maternal inbred lines possessing CMS, and paternal inbred lines possessing fertility-restoring genes sunflower hybrids are developed (Kaya et al. 2012). Hybrids achieve seed yields 25–30% higher than cultivars. Hybrid breeding allows combination of resistance and tolerance from different inbred lines which gives durable resistance/tolerance to a certain constraint. According to Škorić (2016) main abiotic factors in sunflower production are drought, mineral toxicities/deficiencies, and frost. Occupying the first position as the most unfavorable abiotic stress, drought is widespread on over one third of the soils worldwide (Škorić 2016). Because of complexity and polygenic nature of abiotic stress for implementation of appropriate breeding method it is necessary to choose adequate strategy. Regarding drought stress, there are numerous strategies utilized in breeding such as induction of earliness for drought escape, modification of certain plant traits that leads toward drought resistance and introduction of drought tolerant traits associated with high yield (Rauf 2008).

3.5.7 Use of Morphological Markers

Morphological markers have been utilized in plant breeding a long time ago. They served to plant breeders as a tool to evaluate variability and visually distinguish qualities like plant architecture, seed structure, flower colour, growth habit and other important agronomic traits. They are used in traditional breeding as they do not require specific instruments, facilities or qualified technicians. Morphological markers have been successfully used by breeders of various crops and they also play an important role in the management and maintenance of Plant Genetic Resources (PGR), as well as in Plant Breeders' Rights (PBR) system (Babić et al. 2016; Nadeem et al. 2018). They can be used for design of desirable genotype by combination of favorable genes which are identified based on inheritance of the gene in relation to morphological indicators of a particular trait (Kutcher et al. 1996). They are also very important as they have been associated with a number of quantitative traits in determination of the agronomic value and also in taxonomic classification (Jin et al. 1993; Ortiz et al. 2008). Russian farmers were among the first "sunflower breeders "in modern history as they were choosing individual plants based on morphological characteristics like head size, seed size and stay-green characteristic. One of the most significant morphological markers to determine drought tolerant genotypes in sunflower is stay green trait (Škorić 2012, 2016). The use of stay green criteria is also connected with resistance to *Macrophomina* and simultaneously selected inbred lines also showed resistance to *Phomopsis* (Vrânceanu et al. 1992; Škorić 2016). Special attention when using stay green trait should be focused on selection on genotypes with high percent of self-fertility.

Main disadvantages of morphological markers are unknown mechanism of genetic control and they are also limited in number as it depends on the plant growth phase which is also largely influenced by environmental conditions (Eagles et al. 2001; Babić et al. 2016). Morphological markers are often considered unreliable indicators reflected through low level of polymorphism, low heritability, late expression, limited discriminative power and potential to measure relatedness and genetic similarity (Babić et al. 2016).

3.5.8 Use of Molecular Markers

Sunflower breeding for new varieties better adapted to abiotic stresses based on the traditional methods and technologies takes time, money and human effort, though their efficiency is often low (Duca 2008; Debaeke et al. 2017). Molecular markers played a major role in acceleration of that process and identification and introduction of abiotic stress relate genes into elite germplasm (Miladinović et al. 2019). Consequently, molecular markers can be used as valuable tool for identification of parental lines and combinations (Suresha et al. 2017).

Abiotic stress tolerance in sunflower is mostly controlled by several genes, hence breeders need to put a lot of effort in identification and validation of tolerance QTLs that could be used in MAS. Most of the studies deal with drought tolerance related traits. The first study on the identification of drought tolerance QTLs was published by Jamaux et al. (1997), who mapped QTLs by random amplified polymorphic DNA (RAPD), random fragment length polymorphism (RFLP) and sequence-tagged site (STS) bulked analysis. Herve et al. (2001) mapped 19 QTLs associated with water status by use of amplified fragment length polymorphism (AFLP) markers. Detected QTLs described between 8.8 and 62.9% of the phenotypic variance of each examined trait. In addition to QTL mapping, Kiani et al. (2007a) performed expression analysis in determining drought tolerance. Several QTLs were associated with the expression of water status traits and net photosynthesis rate. The same authors Kiani et al. (2007b) analysed agronomical and yield parameters in two water stress conditions in greenhouse and the field and recommended marker from Chap. 14, ORS391, in combination with a marker that is the closest to the common QTL for plant status and osmotic adjustment, ORS523-1, for pyramiding of QTLs that are associated with yield and drought tolerance (Kiani et al. 2009). In their research, Abdi et al. (2012) used SNP-based cleaved amplified polymorphic sequence (CAPS) markers and identified regions that carry colocalized QTLs for several drought tolerance related traits on Chaps. 5, 10, 14 and 17, concluding that markers associated to these detected QTLs could be a valuable tool in marker-assisted breeding. Adiredjo et al. (2014) identified QTLs associated with water use efficiency and carbon isotope discrimination in sunflower leaves and identified the most promising regions for MAS on Chaps. 6 and 13 that carried QTLs associated to examined traits, of which carbon isotope discrimination was found to be as the most important one and in high negative correlation with WUE.

One of the rare molecular studies of sunflower reaction to low temperature was done by Allinne et al. (2009). The authors detected several putative genomic regions involved in the variation of sunflower physiological traits under low temperature. They recommended the major QTLs for cold tolerance associated with SSR markers, such as ORS331-2, for the cell membrane stability, to be checked in several environments to see if they can be used in marker-assisted selection programs. Since sunflower adaptation to individual and combined stress is imparted through a complex, yet to be fully understood mechanisms, meta-analysis could be a useful tool for study of molecular mechanism behind multiple individual stress and combined stress tolerance in sunflower (Shaik and Ramaskrishna 2013). Under combined or multiple stresses, the meta-analysis can also be used for identification of candidate genes for multiple and combined stress tolerance (Ramu et al. 2016).

3.6 Limitations of Traditional Breeding and Prospects of Genomic Designing

Publishing of sunflower genome sequence provided the foundation for exploitation of genetic diversity and wider use of genomic and other—omics tools in sunflower breeding (Badouin et al. 2017). Furthermore, recent breakthroughs in genome editing (GE) technologies have opened a new era in plant breeding and paved the way for introduction of precision breeding in sunflower breeding programmes, by providing more precise and efficient tools to increase sunflower abiotic stress resilience by means of trait engineering (Miladinović et al. 2021).

3.6.1 Integration of—Omics Technologies

Sunflower is considered tolerant to abiotic stress, hence there are not genomics studies related to its reaction to abiotic stress, since most of the papers are focused on biotic stress resistance and improved quality. Several studies dealt with transcriptomics of sunflower response to different abiotic stresses. In the most recent study, Gody et al. (2020) provided transcriptomic data of sunflower leaves subjected to water deficit, that differentiated both plant water status and the different genotypes. Ramu et al. (2016) studied combined response of sunflower plants to different stresses using meta-analysis of publicly available transcriptome data. The authors found that menadione-based screening can be used for identification both of genotypes tolerant to different biotic and abiotic stresses, and the genotypes tolerant to combined stresses. In their work, Balliau et al. (2021) studied proteomic response of sunflower plants to water deficit. They have identified 3062 proteins and the quantified 1211 of them in the leaves of the 24 sunflower genotypes grown under two watering regimes, hence producing data that could be of use in the study of the effects of genotype and watering conditions on sunflower proteome. A combination of targeted and untargeted metabolomic analyses on water-stressed and fully irrigated sunflower leaf samples was used for characterization of metabolic markers for discriminating sunflower genotypes and environmental conditions (Fernandez et al. 2019). The results obtained indicated that only limited number of metabolic markers can clearly differentiate samples under different stresses in a more discriminant manner than classical physiological data used to discriminate individuals subjected to water stress.

Immense progress made in the field of—omics have opened new paths to understand and study the mechanisms of abiotic stress tolerance in plants (Ozturk Gokce et al. 2020). Since using only one—omics approach is not sufficient to develop novel abiotic stress resistant crop varieties, efficient combination of different -omic tools and techniques looks like a promising strategy (Chaudhary et al. 2019). The same stands for sunflower, where the integration of various—omics approaches is a prerequisite for elucidation of the complex abiotic stress response. The first, and up to our knowledge, the only work using integrated approach to study sunflower response to water-deficit conditions was described by Sarazin et al. (2017) who used genomics (RNA-seq and quantitative RT-PCR), physiological (growth, water statute, stomatal conduction evaluations, and transpiration rate) and biochemical analyses (LC–MS). The authors concluded that sunflower water stress tolerance is correlated with a transcriptome fine-tuning that triggers activation of ABA-dependent genes and not to ABA overproduction.

3.6.2 Genomic Studies

Exploitation of available genetic resources in combination with different genomic tools could lead to considerable improvements in sunflower resilience to abiotic stresses. For instance, Gao et al. (2019) used genome-wide association study (GWAS) for analysis of variation of sunflower crop performance and response to flooding (Gao et al. 2019). The authors identified a subset of loci conferring flooding resistance without negative effects on plant growth and concluded that genomic selection (GS) could be efficiently used for the development of cultivars that are both flooding-tolerant and highly productive. The first high throughput sequencing study for gene expression profiling analysis of sunflower under drought stress has been performed by Liang et al. (2017). The authors have identified 17 genes that play roles in sunflower response to abiotic stress and may be relevant for drought tolerance, as well. The results of their study also demonstrated the complex nature of the drought stress response in sunflower, involving multiple metabolic pathways.

GS uses genome-wide molecular data as predictors of performance of genotype under certain stress conditions and enables development of quantitative trait loci (Würschum et al. 2013; Fillipi et al. 2014). GS develops the prediction model by integration of genotypic and phenotypic data of training population which are used to obtain genomic estimated breeding values (GEBVs) (Meuwissen et al. 2001). GS so far has not been used for prediction of abiotic stress tolerance in sunflower, but for the prediction of hybrid performance (Reif et al. 2013), hybrid oil content (Mangin et al. 2017) and *Sclerotinia* mid-stalk rot tolerance (Livaja et al. 2016). Nevertheless, the results obtained in these studies showed that GS could improve breeding efficiency, especially in the cases when either one or both parents are not well-characterized. Besides that, these first steps in genomic selection showed that it can successfully address complex quantitative traits in sunflower, one related to abiotic stress tolerance included (Dimitrijević and Horn 2018).

3.6.3 Use of Modern Breeding Techniques

There have been several attempts to introduce genes for abiotic stress resistance into sunflower using different genetic transformation methods. Watanabe et al. (2005) incorporated yeast Metallothionein metallothionein gene (*CUP1*) into sunflower and succeeded to select heavy metal-tolerant sunflower calluses thus confirming successful transfer of the trait. Cheng et al. (2009) inserted the drought and salt resistance gene *P5CS* into sunflower and obtained six transformed buds but did not manage to obtain fertile transgenic plants. Tishchenko et al. (2014) introduced dsRNA-suppressor of proline dehydrogenase gene into sunflower plants with the aim to increase their tolerance to water deficiency and salinity. However, due to lack of commercial interest and regulatory issues, up to date, no application for the market introduction of a genetically modified sunflower has been made (ISAAA 2021).

Development of GE tools and techniques, could provide new perspectives for more efficient sunflower breeding for complex phenotypic traits controlled by polygenes, including traits related to abiotic stress resistance. Up to date, there is only one report on use of GE in sunflower. Ynet and Yilancioglu (2018) described CRISPR/Cas9 model for sunflower resistance for biotic and abiotic stresses. The authors determined the suitable genetic changes to perform and design of relevant plasmids, plasmid transfer method into the sunflower genome, as well as the control for whether the plasmid is transferred into the genome and if GE is effective.

Both in genetic transformation and GE, regeneration efficiency could be an obstacle for their effective use in sunflower breeding. In order to overcome this problem, Zhang (2016) proposed the use of low inoculum with long coculture (LI/LC) transformation protocol, while Ikeda et al. (2005) used small and branching varieties of sunflower in order to increase plant regeneration and gene introduction.

Combined with the classical genetic studies, this newly available genome sequence, along with the advanced sequencing technologies could enable the study of the epigenetic phenomena in sunflower and the application of epigenome profiling and engineering for creation of the genotypes with the durable abiotic stress resilience (Varotto et al. 2020). Advances in—omics research provided new tools and models for understanding of abiotic stress related traits and to identify target traits useful for sunflower improvement. They opened the way to the discovery of loci affecting sunflower reaction to stress conditions or the expression of stress tolerance related traits, as well as identification of genes that could be useful either as candidate sequences for QTLs or for further manipulation using modern breeding tools, also useful for the removal of detrimental mutations or enrichment of specific sequences with either alleles from wild relatives or other abiotic stress resistance sources (Anđelković et al. 2020).

Acknowledgements This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-9/2021-14/200032.

References

Abdi N, Darvishzadeh R, Jafari M, Pirzad A, Haddadi P (2012) Genetic analysis and QTL mapping of agro-morphological traits in sunflower (*Helianthus annuus* L.) under two contrasting water treatment conditions. Plant Omics 5(2):149

Acquaah G (2015) Conventional plant breeding principles and techniques. In: Al-Khayri J, Jain S, Johnson D (eds) Advances in plant breeding strategies: breeding, biotechnology and molecular tools. Springer, Cham

Adiredjo AL, Navaud O, Munos S, Langlade NB, Lamaze T, Grieu P (2014) Genetic control of water use efficiency and leaf carbon isotope discrimination in sunflower (*Helianthus annuus* L.) subjected to two drought scenarios. PLoS One 9(7):e101218

Ahmad S, Raza I, Ali H, Shahzad AN, Atiq-ur-Rehman SN (2014) Response of cotton crop to exogenous application of glycine betaine under sufficient and scarce water conditions. Braz J Bot 37:407–415

Al-Khatib K, baumgartner JR, Currie RS (1999) Survey of common sunflower (Helianthus annuus) resistance to ALS inhibiting herbicides in northeast Kansas. In: Proceedings of 21th sunflower research workshop. National Sunflower Assocication, Bismark, N.D., pp 210–215

Al-Khatib K, Miller JF (2000) Registration of four genetic stocks of sunflower resistant to imidazolinone herbicide. Crop Sci 40:869–870

Allinne C, Maury P, Sarrafi A, Grieu P (2009) Genetic control of physiological traits associated to low temperature growth in sunflower under early sowing conditions. Plant Sci 177:349–359

Alonso LC, Rodriguez-Ojeda MI, Fernandez-Escobar J, Lopez-Ruiz-Calero G (1998) Chemical control of broomrape (*Orobanche cernua* Loefl.) in sunflower (*Helianthus annuus* L.) resistant to imazethapyr herbicide. Helia 21(29):45–54

Amanullah A, Khan MW (2010) Interactive effects of potassium and phosphorus on phenology and grain yield of sunflower in northwest Pakistan. Pedosphere 20:674–680

Anđelković V, Cvejić S, Jocić S, Kondić-Špika A, Marjanović Jeromela A, Mikić S, Prodanović S, Radanović A, Savić Ivanov M, Trkulja D, Miladinović D (2020) Use of plant genetic resources in crop improvement—example of Serbia. Gen Resour Crop Evol 67:1935–1948

Andre E (2004) Drought effects on growth stages and yield components of sunflower hybrids. In: Proceedings of the 16th international sunflower conference, Fargo, ND, USA, 29. August-4 September 2004. Intl Sunflower Assoc, Paris, France 1:251–255

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3–16

Babić V, Nikolić A, Andjelković V, Kovačević D, Filipović M, Vasić V, Mladenović-Drinić S (2016) A UPOV morphological versus molecular markers for maize inbred lines variability determination. Chil J Agric Res 76(4):417–426

Badouin H, Gouzy J, Grassa CJ, Murat F, Staton SE, Cottret L, Lelandais-Brière C, Owens GL, Carrère S, Mayjonade B, Legrand L et al (2017) The sunflower genome provides insights into oil metabolism, flowering and asterid evolution. Nature 546:148–215

Baldini M, Vannozzi GP (1998) Agronomic and physiological assessment of genotypic variation for drought tolerance in sunflower genotypes obtained from a cross between *H. annuus* and *H. argophyllus*. Agri Med 128:232–240

Baldini M, Vannozzi GP (1999) Yield relationships under drought in sunflower genotypes obtained from a wild population and cultivated sunflowers in rain-out shelter in large pots and field experiments. Helia 222:81–96

Baldini M, Cecconi F, Vannozzi GP (1993) Influence of water deficit on gas exchange and dry matter accumulation in sunflower cultivars and wild species (*Helianthus argophyllus* T. & G). Helia 16:1–10

Baligar VC, Fageria NK, He ZL (2001) Nutrient use efficiency in plants. Commun Soil Sci Plant Anal 32(7–8):921–950

Balliau T, Duruflé H, Blanchet N, Blein-Nicolas M, Langlade NB, Zivy M (2021) Proteomic data from leaves of twenty-four sunflower genotypes under water deficit. OCL 28:12

Barkley NA, Wang M (2008) Application of tilling and eco-tilling as reverse genetic approaches to elucidate the function of genes in plants and animals. Curr Gen 9:212–226

Barros JF, de Carvalho M, Basch G (2004) Response of sunflower (*Helianthus annuus* L.) to sowing date and plant density under Mediterranean conditions. Eur J Agron 21:347–356

Basseto MA, Ceresini PC, Valério Filho WV (2007) Severidade da mela da soja causada por *Rhizoctonia solani* AG-1 IA emfunção de doses de potássio. Summa Phytopathol 33(1):56–62

Belhassen E, Castiglioni VPR, Chimenti C, Griveau Y, Jamaux I, Steinmetz A (1996) Looking for physiological and molecular markers of leaf cuticular transpiration using interspecific crosses between *Helianthus argophyllus* and *Helianthus annuus*. ISA Symposium II: Drought Tolerance in Sunflower. Beijing. PR China. 14 June 1996, pp 39–45

Blamey FPC, Asher CJ, Edwards DG (1987) Hydrogen and aluminium tolerance. In: Proceedings of the second international symposium on genetic aspects of plant mineral nutrition. University of Wisconsin, Madison, pp 16–20

Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Austral J Agric Res 56:1159–1168

Bowsher AW, Milton EF, Donovan LA (2016) Comparison of desert-adapted *Helianthus niveus* (Benth.) Brandegee ssp. tephrodes (A. Gray) Heiser to cultivated *H. annuus* L. for putative drought avoidance traits at two ontogenetic stages. Helia 39(64):1–19

Božić D, Sarić M, Malidža G, Ritz C, Vrbničanin S (2012) Resistance of sunflower hybrids to imazamox and tribenuron-methyl. Crop Protec 39:1–10

Bruniard JM, Miller JF (2001) Inheritance of imidazolinone herbicide resistance in sunflower. Helia 24:11–16

Cadic E, Coque M, Vear F, Grezes-Besset B, Pauquet J, Piquemal J, Lippi Y, Blanchard P, Romestant M, Pouilly N, Rengel D (2013) Combined linkage and association mapping of flowering time in sunflower (*Helianthus annuus* L.). Theor Appl Genet 126(5):1337–1356

Canavar Ö, Götz KP, Ellmer F, Chmielewski FM, Kaynak MA (2014) Determination of the relationship between water use efficiency, carbon isotope discrimination and proline in sunflower genotypes under drought stress. Austral J Crop Sci 8:232–242

Cellier F, Ouvrard O, Ferrare K, Tousch D, Lamaze T, Dupuis, JM, Casse-Delbart F (1996) Differential expression of water stress-regulated genes in drought tolerant or sensitive sunflower genotypes. ISA-Symposium II: Drought Tolerance in Sunflower. Beijing. PR China, 14 June 1996, pp 36–39

Ceyhan E, Onder M, Oztür O, Harmankaya M, Hamurcu M, Gezgin S (2008) Effects of application boron onyields, yield component and oil content of sunflower in boron-deficient calcareous soils. Afr J Biotechnol 7(16):2854–2861

Chandler JM, Jan CC (1984) Identification of salt-tolerant germplasm source in the *Helianthus species.* Agron Abstracts p 61

Chaudhary J, Khatri P, Singla P, Kumawat S, Kumari A, Vikram A, Jindal SK, Kardile H, Kumar R, Sonah H, Deshmukh R (2019) Advances in omics approaches for abiotic stress tolerance in tomato. Biology 8:90

Cheng JD, An YL, Sun RF (2009) Drought and salt-alkali resistance gene *P5CS* transformed into sunflower inbred lines. Biotechnol Bull 3:65–69

Cheres MT, Knapp SJ (1998) Ancestral origins and genetic diversity of cultivated sunflower: coancestry analysis of public germplasm. Crop Sci 38:1476–1482

Chiementi CA, Pearson J, Hall AJ (2002) Osmotic adjustment and yield maintenance under drought in sunflower. Field Crops Res 75:235–246

Chimenti C, Hall A (2001) Grain number responses to temperature during floret differentiation. Field Crop Res 72:177–185

Chimenti C, Hall A, Lopez M (2001) Embryo-growth rate and duration in sunflower as affected by temperature. Field Crop Res 69:81–88

Chimenti C, Giuliano J, Hall A (2004) Osmotic adjustment, its effects on yield maintenance under drought in sunflower. In: Proceedings of the 16th international sunflower conference, Fargo, ND, USA, 29 Aug-4 Sept 2004, vol 1, Intl. Sunflower Assoc., Paris, France, pp 261–267

Christopher JT, Christopher MJ, Jennings R, Jones S, Fletcher S, Borrell AK, Manschadi AM, Jordan D, Mace E, Hammer GL (2013) QTL for root angle and number in a population developed from bread wheats (*Triticum aestivum*) with contrasting adaptation to water-limited environments. Theor Appl Genet 126:1563–1574

Cicek N, Pekcan V, Arslan O, Erdal SC, Nalcaiyi ASB, Cil AN, Sahin V, Kaya Y, Ekmekci Y (2019) Assessing drought tolerance in field-grown sunflower hybrids by chlorophyll fluorescence kinetics. Braz J Bot 42:249–260

Comas LH, Becker SR, Cruz VM, Byrne PF, Dierig DA (2013) Root traits con-tributing to plant productivity under drought. Front Plant Sci 4:442

Conaty WC, Tan DKY, Constable GA, Sutton BG, Field DJ, Mamum EA (2008) Genetic variation for waterlogging tolerance in cotton. J Cotton Sci 12:53–61

Cooper MFA, van Eeuwijk GL, Hammer DW, Podlich C, Messina (2009) Modelling QTL for complex traits: Detection and context for plant breeding. Curr Opin Plant Biol 12:2:231–240

Coque M Mesnildrey S, Romestant M, Grezes-Besset B, Vear F, Langlade N, Vincourt P (2008) Sunflower line core collections for association studies and phenomics. In: Proceeding of the 17th International Sunflower Conferences, 8–12 June 2008, Cordoba, Spain, pp 725–728

Courtois B, Ahmadi N, Khowaja F, Price AH, Rami JF, Frouin J, Hamelin C, Ruiz M (2009) Rice root genetic architecture: meta-analysis from a drought QTL database. Rice 2:115–128

Cukadar-Olmedo B, Miller JF (1997) Inheritance of the stay green trait in sunflower. Crop Sci 37:150–153

Cvejić S, Miladinović D, Jocić S (2014) Mutation breeding for changed oil quality in sunflower. In: Tomlekova NB, Kozgar MI, Wani MR (eds) Mutagenesis: exploring genetic diversity of crops. Wageningen Academic Publishers, Wageningen, Netherlands, pp 77–96

Cvejić S, Jocić S, Malidža M, Radeka I, Jocković M, Miklič V, Stojanović D (2016) New sunflower hybrids tolerant to tribenuron-methyl. Plant Breeding and Seed Production 22(2):61–68

Cvetkova F (1970) Initial material for breeding by gamma and X irradiation. Genet Plant Breed 3:231–237

de Dorlodot S, Forster BP, Pages L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trend Plant Sci 12(10):474–481

de la Vega AJ, Chapman SC (2010) Mega-environment differences affecting genetic progress for yield and relative value of component traits. Crop Sci 50:574–583

de la Haba P, de la Mata L, Molina E, Agüera E (2014) High temperature promotes early senescence in primary leaves of sunflower (*Helianthus annuus* L.) plants. Can J Plant Sci 94:659–669

Debaeke P, Casadebaig P, Flenet F, Langlade N (2017) Sunflower crop and climate change: vulnerability, adaptation, and mitigation potential from case-studies in Europe. OCL 24(1):D102

Demir AO, Goksoy AT, Buyukcangaz H, Turan ZM, Koksal ES (2006) Deficit irrigation of sunflower (*Helianthus annuus* L.) in a sub-humid climate. Irrig Sci 24:279–289

Di Caterina R, Giuliani MM, Rotunno T, De Caro A, Flagella Z (2007) Influence of salt stress on seed yield and oil quality of two sunflower hybrids. Ann Appl Biol 151:145–154

Dimitrijević A, Horn R (2018) Sunflower hybrid breeding: from markers to genomic selection. Front Plant Sci 8:2238

Ding TL, Yang Z, Wei X, Yuan F, Yin S, Wang B (2018) Evaluation of salt-tolerant germplasm and screening of the salt-tolerance traits of sweet sorghum in the germination stage. Funct Plant Biol 45(10):1073–1081

Duca M (2008) Genetic—phytohormonal interactions in male fertility and male sterility phenotype expression in sunflower (*Helianthus annuus* L.). Communication 1. Comparative investigation of auxin and gibberellin quantities in diverse sunflower genotypes. Helia 32(48):27–38

Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ (2001) Implementation of markers in Australian wheat breeding. Aust J Agric Res 52:11–12

Ebrahimi A, Maury P, Berger M, Poormohammad Kiani S, Nabipour A, Shariati F, Grieu P, Sarrafi A (2008) QTL mapping of seed-quality traits in sunflower recombinant inbred lines under different water regimes. Genome 51:599–615

Edelist C, Raffoux X, Falque M, Dillmann C, Sicard D, Rieseberg LH, Karrenberg S (2009) Differential expression of candidate salt-tolerance genes in the halophyte *Helianthus paradoxus* and its glycophyte progenitors *H. annuus* and *H. petiolaris* (Asteraceae). Amer J Bot 96:1830–1838

El Midaoui M, Serieys H, Griveau Y, Benbella M, Talouizte A, Berville A, Kaan F (2003) Effects of osmotic and water stresses on root and shoot morphology and seed yield in sunflower (*Helianthus annuus* L.) genotypes bred for Morocco or issued from introgression with *H. argophyllus* T. & G. and *H. debilis* Nutt. Helia 26:1–16

El-Soda M, Malosetti M, Zwaan BJ, Koornneef M, Aarts MGM (2014) Genotype × environment interaction QTL mapping in plants: lessons from *Arabidopsis*. Trend Plant Sci 19:390–439

Encheva J, Shindrova P, Penchev E (2008) Developing mutant sunflower lines (*Helianthus annuus* L.) through induced mutagenesis. Helia 31(48):61–72

Encheva J, Shindrova P (2011) Developing mutant sunflower lines (*Helianthus annuus* L.) through induced mutagenesis and study of their combining ability. Helia 34:107–122

Fabie A, Miller JF (2002) Cross-resistance of two sulfonylurea-resistant sunflower sources to selected ALS herbicides. In: Proceeding of the 24th sunflower research workshop, Fargo, 17–18 Jan 2002, pp 117–122

FAO (2017) FAOSTAT

Fernandez O, Urrutia M, Berton T, Bernillon S, Deborde C, Jacob D, Maucourt M, Maury P, Duruflé H, Gibon Y, Langlade NB, Moing A (2019) Metabolomic characterization of sunflower leaf allows discriminating genotype groups or stress levels with a minimal set of metabolic markers. Metabolomics 15:56

Fernández-Martínez JM, Pérez-Vich B, Velasco L (2009) Sunflower. In: Vollmann J, Rajčan I (eds) Oilcrops (handbook of plant breeding). Springer, New York, pp 155–232

Filippi C, Aguirre N, Rivas JG, Zubrzycki J, Puebla A, Cordes D, Moreno MV, Fusari CM, Alvarez D, Heinz RA et al (2015) Population structure and genetic diversity characterization of a sunflower association mapping population using SSR and SNP markers. BMC Plant Biol 15:52

Fillipi C, Zubrzycki J, Lia V, Heinz RA, Paniego NB, Hopp HE (2014) Genetics and genomics applied to sunflower breeding. In: Sunflowers: Growth and Development, Environmental Influences and Pests/Diseases, Arribas J.I. (ed). Nova Science Publisher, pp 61-94

Filippi CV, Merino GA, Montecchia JF, Aguirre NC, Rivarola M, Naamati G, Fass MI, Álvarez D, Di Rienzo J, Heinz RA, Contreras Moreira B, Lia VV, Paniego NB (2020) Genetic diversity, population structure and linkage disequilibrium assessment among international sunflower breeding collections. Genes 11(3):283

Flagella Z, Giuliani MM, Rotunno T, Di Caterina R, De Caro A (2004) Effect of saline water on oil yield and quality of a high oleic sunflower (*Helianthus annuus* L.) hybrid. Europ J Agron 21:267–272

Gao Y, Lynch JP (2016) Reduced crown root number improves water acquisition under water deficit stress in maize (*Zea mays* L.). J Exp Bot 67:4545–4557

Gao L, Lee JS, Hübner S, Hulke BS, Qu Y, Rieseberg LH (2019) Genetic and phenotypic analyses indicate that resistance to flooding stress is uncoupled from performance in cultivated sunflower. New Phytol 223:1657–1670

García-Vila M, Fereres E, Prieto MH, Ruz C, Soriano MA (2012) Sunflower. In: Steduto P, Hsiao TC, Fereres E, Raes D (eds) Crop yield response to water FAO, Rome, p 164–173

Gody L, Duruflé H, Blanchet N, Carré C, Legrand L, Mayjonade B, Muños S, Pomiès L, de Givry S, Langlade NB, Mangin B (2020) Transcriptomic data of leaves from eight sunflower lines and their sixteen hybrids under water deficit. OCL 27:48

Goebl AM, Kane NC, Doak DF, Rieseberg LH, Ostevik KL (2020) Contrasting selection at multiple life stages maintains divergent adaptation between 3 sunflower ecotypes. bioRxiv. https://doi.org/10.1101/2020.08.08.242503

Gornik K (2011) The effect of temperature treatments during 'Wielkopolski' sunflower seed imbibition and storage on plant tolerance to chilling. Folia Hort 23(1):83–88

Grassini P, Indaco GV, Pereira ML, Hall AJ, Trápani N (2007) Responses to short-term waterlogging during grain filling in sunflower. Field Crop Res 101(3):352–363

Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156:1–13

Harter AV, Gardner KA, Falush D, Lentz DL, Bye RA, Rieseberg LH (2004) Origin of extant domesticated sunflowers in eastern North America. Nature 430:201–205

Hatfield JL, Dold C (2019) Water-use efficiency: advances and challenges in a changing climate. Front Plant Sci 10:103

Hepler PK, Vidali L, Cheung AY (2001) Polarized cell growth in higher plants. Annu Rev Cell Develop Biol 17:159–187

Herve D, Fabre F, Berrios EF, Leroux N, Chaarani GA, Planchon C, Sarrafi A, Gentzbittel L (2001) QTL analysis of photosynthesis and water status traits in sunflower (*Helianthus annuus* L.) under greenhouse conditions. J Exp Bot 52(362):1857–1864

Hewezi T, Leger M, El Kayal W, Gentzbittel L (2006) Transcriptional profiling of sunflower plants growing under low temperatures reveals an extensive down-regulation of gene expression associated with chilling sensitivity. J Exp Bot 57(12):3109–3122

Hladni N (2010) Genes and Sunflower Yield. Andrejević Foundation, Belgrade, Serbia

Hladni N, Miladinović D (2019) Confectionery sunflower breeding and supply chain in Eastern Europe. OCL 26:29

Hladni N, Terzić S, Mutavdžić B, Zorić M (2017) Classification of confectionary sunflower genotypes based on morphological characters. J Agric Sci 155:1594–1609

Hladni N, Zorić M, Terzić S, Ćurčić N, Šatović Z, Perović D, Panković D (2018b) Comparison of methods for the estimation of best parent heterosis among lines developed from interspecific sunflower germplasm. Euphytica 214:108

Hladni N, Miladinović D, Jocić S, Mikilč V, Marjanović Jeromela A (2018a) The use of crop wild relatives in sunflower breeding for drought response. In: Proceedings of international symposium—sunflower and climate change. Toulouse, France, 5–6 Feb 2018

Hniličková H, Hejnák V, Němcová L, Martinková J, Skalický M, Hnilička F, Grieu P (2017) The effect of freezing temperature on physiological traits in sunflower. Plant Soil Environ 63:375–380

Homann MJ (2017) Auswirkungen des Klimawandels auf atmosphärische Zirkulations typhäufigkeiten und starke Gebietsnie derschlägeims üdlichen Mitteleuropa. Dissertation. Universität Augsburg, Augsburg, Germany, pp 1–154

Hu J, Vick BA (2003) Target region amplification polymorphism: a novel marker technique for plant genotyping. Plant Mol Biol Rep 21:289–294

Hu W, Jiang N, Yang J, Meng Y, Wang Y, Chen B et al (2016) Potassium (K) supply affects K accumulation and photosynthetic physiology in two cotton (*Gossypium hirsutum* L.) cultivars with different K sensitivities. Field Crop Res 196:51–63

Huang K, Andrew RL, Owens GL, Ostevik KL, Rieseberg LH (2020) LH Multiple chromosomal inversions contribute to adaptive divergence of a dune sunflower ecotype. Mol Ecol 29:2535–2549

Hübner S, Bercovich N, Todesco M, Mandel JR, Odenheimer J, Ziegler E, Lee JS, Baute GJ, Owens GL, Grassa CJ et al (2019) Sunflower pan-genome analysis shows that hybridization altered gene content and disease resistance. Nat Plant 5:54–62

Hussain S, Ahmad M, Ahmad S, Iqbal J, Subhani MN, Nadeem SM, Atta S, Ibrahim M. (2013) Improvement of drought tolerance in sunflower (*Helianthus annuus* L.) by foliar application of abscisic acid and potassium chloride. Pak J Nutr 12(4):345–352

Hussain MK, Rehman OU (1993) Breeding sunflower for salt tolerance: physiological basis for salt tolerance in sunflower (*Helianthus annuus* L.). Helia 16:77–84

Hütsch BW, Schubert S (2017) Harvest index of maize (*Zea mays* L.). Are there possibilities for improvement? In: Sparks DL (ed) Advances in agronomy. 146:37–82

Ikeda M, Matsumura M, Kamada H (2005) Suitability of small and branching sunflower varieties for molecular genetic experiments and their transformation by *Agrobacterium* infection. Plant Biotechnol 22:97–104

ISAAA (2021) The international service for the acquisition of agri-biotech applications. USA. http://www.isaaa.org/. Assessed 25 Feb 2021

Jabeen N, Ahmad R (2012) Improving tolerance of sunflower and safflower during growth stages to salinity through foliar spray of nutrient solutions. Pak J Bot 44(2):563–572

Jamaux I, Steinmetz A, Belhassen E (1997) Looking for molecular and physio-logical markers of osmotic adjustment in sunflower. New Phytol 137(1):117–127

Janila P, Variath MT, Pandey MK, Desmae H, Motagi BN, Okori P, Manohar SS, Rathnakumar AL, Radhakrishnan T, Liao B, Varshney RK (2016) Genomic tools in groundnut breeding program: status and perspectives. Front Plant Sci 7:289

Javaid T, Bibi A, Sadaqat HA, Javed S (2015) Screening of sunflower (*Helianthus annuus* L.) hybrids for drought tolerance at seedling stage. Intl J Plant Sci Ecol 1(1):6–16

Jin Y, Statler GD, Franckowiak JD, Steffenson BJ (1993) Linkage between leaf rust resistance genes and morphological markers in barley. Phytopathol 83:203–233

Jocić S, Malidža G, Cvejić S, Hladni N, Miklič V, Škorić D (2011) Development of sunflower hybrids tolerant to tribenuron methyl. Genetika 43(1):175–182

Jocić S, Miladinović D, Kaya Y (2015) Breeding and genetics of sunflower. In: Martínez-Force E, Dunford NT, Salas JJ (eds) Sunflower: chemistry, production, processing, and utilization. AOCS Press, Urbana, IL, pp 1–26

Jocić S, Miklič V, Malidža G, Hladni N, Gvozdenović S (2008) New sunflower hybrids tolerant of tribenuron-methyl. In: Proceeding of the 17th international sunflower conference. Cordoba, Spain, 8–12 June 2008, pp 505–508

Jocković M, Cvejić S, Jocić S, Marjanović Jeromela A, Miladinović D, Jocković B, Miklič V, Radić V (2019) Evaluation of sunflower hybrids in multi-environment trial (MET). Turk J Field Crops 24(2):202–210

Jursík M, Hamouzova K, Soukup J, Andr J, Holec J (2015) Differences in sensitivity of F1 and F2 generations of herbicide tolerant sunflower volunteers to selected acetolactate synthase inhibiting herbicides. Plant Soil Environ 60(10):446–451

Kalaydzhyan AA, Khlevnoy LV, Neshchadim NN, Golovin VP, Vartanyan VV, Burdun AM (2007) Rossiyskiysolnechnyytsvetok. Krasnodar, Sovet. Kuban. Russia, p 342 (In Russian)

Kalaydzhyan AA, Neshchadim NN, Osipyan VO, Škorić D (2009) Kuban sunflower gift to the world. Monograph. Ministry of Russian Agriculture—Russian Academy of Agriculture-Kuban State Agrarian University, Krasnodar. Russia, p 498 (In Russian)

Kalyar T, Rauf S, Teixeira da Silva JA, Haidar S, Iqbal Z. (2013a) Utilization of leaf temperature for selection of leaf gas exchange traits for the induction of heat resistance in sunflower (*Helianthus annuus* L.). Photosynthetica 51(3):419–428

Kalyar T, Rauf S, Teixeira da Silva JA, Shahzad M (2013b) Handling sunflower (*Helianthus annuus* L.) populations underheat stress. Arch Agron Soil Sci 60(55):655–672

Kalyar T, Rauf S, Teixeira SJA (2014) Handling sunflower (*Helianthus annuus* L.) populations under heat stress. Arch Agron Soil Sci 60:655–672. https://doi.org/10.1080/03650340.2013.799276

Kantar MB, Sosa CC, Khoury CK, Castañeda-Álvarez NP, Achicanoy HA, Bernau V, Kane NC, Marek L, Seiler G, Rieseberg LH (2015) Ecogeography and utility to plant breeding of the crop wild relatives of sunflower (*Helianthus annuus* L.). Front Plant Sci 6:1–11

Katerji N, Van Hoorn JW, Hamdy A, Karam F, Mastroruilli M (1994) Effect of salinity on emergence and on water stress and early seedling growth of sunflower and maize. Agric Wat Mange 26:81–91

Kaya Y, Balalić I, Miklič V (2015) Eastern Europe perspectives on sunflower production and processing. In: Force EM, Dunford NT, Salas JJ (eds) Sunflower chemistry, production, processing, and utilization. AOCS Press, Urbana, Illinois USA, pp 617–637

Kaya Y, Jocic S, Miladinovic D (2012). Sunflower. In Technological Innovations in Major World Oil Crops, Springer, New York

Kaya Y (2016) Sunflower In: Gupta SK (ed) Breeding oilseed crops for sustainable production, 1st Edition Opportunities and Constraints. Academic Press, San Diego, pp 55–88

Keipp K, Hütsch BW, Schubert S (2019) How does the harvest index affect water-use efficiency and nutrient-utilization efficiency of sunflowers (*Helianthus annuus* L.)? J Agron Crop Sci 205:519–532

Keipp K, Hutch BW, Ehlers K, Schubert S (2020) Drought stress in sunflower causes inhibition of seed filling due to reduced cell-extension growth. J Agron Crop Sci 206:517–528

Kempel A, Schadler M, Chrobock T, Fischer M, van Kleunen M (2011) Trade-offs associated with constitutive and induced plant resistance against herbivory. Proc Natl Acad Sci USA 108:5685–5689

Khoury CK, Achicanoy HA, Bjorkman AD, Navarro-Racines C, Guarino L, Flores-Palacios X, Engels JMM, Wiersema JH, Dempewolf H, Sotelo S, Ramírez-Villegas J, Castañeda-Álvarez NP, Fowler C, Jarvis A, Rieseberg LH, Struik PC (2016) Origins of food crops connect countries worldwide. Proc Roy Soc B 283:20160792

Kiani SP, Grieu P, Maury P, Hewezi T, Gentzbittel L, Sarrafi A (2007a) Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower (*Helianthus annuus* L.). Theor Appl Genet 114:193–207

Kiani SP, Talia P, Maury P, Grieu P, Heinz R, Perrault A, Nishinakamasu V, Hopp E, Gentzbittel L, Paniego N, Sarrafi A (2007b) Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. Plant Sci 172:773–787

Kiani SP, Maury P, Nouri L, Ykhlef N, Grieu P, Sarrafi A (2009) QTL analysis of yield related traits in sunflower under different water treatments. Plant Breed 128(4):363–373

Kinman ML (1970) New developments in the USDA and state experiment station sunflower breeding programs. In: Proceeding of the fourth international sunflower conference. Memphis, Tenn, pp 181–183

Kolkman JM, Slabaugh MB, Bruniard JM, Berry S, Bushman BS, Olungu C, Maes N, Abratti G, Zambelli A, Miller JF (2004) Acetohydroxyacid synthase mutations conferring resistance to imidazolinone or sulfonylurea herbicides in sunflower. Theor Appl Genet 109:1147–1159. https://doi.org/10.1007/s00122-004-1716-7

Koziol L, Rieseberg LH, Kane N, Bever JD (2012) Reduced drought tolerance during domestication and the evolution of weediness results from tolerance-growth trade-offs. Evolution 66:3803–3814

Kulundžić M, Kovačević A, Viljevac J, Vuletić M, Josipović A, Liović I, Mijić A, Lepeduš H, Kočar M (2016) Impact of abiotic stress on photosynthetic efficiency and leaf temperature in sunflower. Agriculture 22:17–22

Kumar S, Rakshit S, Gupta S (2003) Genetics and cytogenetics of chickpea. In: Ali M, Kumar S, Singh NB (eds) Chickpea Research in India. IIPR, Kanpur, India, pp 31–37

Kusmec A, Srinivasan S, Nettleton D, Schnable PS (2017) Distinct genetic architectures for phenotype means and plasticities in *Zea mays*. Nat Plant 3:715

Kutcher HR, Bailey KL, Rossnagel BG, Franckowiak JD (1996) Linked morphological and molecular markers associated with common root rot reaction in barley. Can J Plant Sci 76:879–883

Lai Z, Livingstone K, Zou Y, Church SA, Knapp SJ, Andrews J, Rieseberg LH (2005) Identification and mapping of SNPs from ESTs in sunflower. Theor Appl Genet 111(8):1532–1544

Lambrides CJ, Chapman SC, Shorter R (2004) Genetic variation for carbon isotope discrimination in sunflower: association with transpiration efficiency and evidence for cytoplasmic inheritance. Crop Sci 44:1642–1653

Leclercq P (1969) Une stérilité cytoplasmique chez le tournesol. Ann Amélior Plant 19:99–106

Leclercq P (1985) Dwarf sunflowers. In: Fernandez-Martinez (ed) Proceedings of the sixth meeting of Eucarpia section of oil and protein. Cordoba, Spain, pp 61–62

Lenssen JPM, Van De Steeg HM, De Kroon H (2004) Does disturbance favour weak competitors? Mechanisms of changing plant abundance after flooding. J Veg Sci 15:305–314

Lexer C, Welch ME, Durphy JL, Rieseberg LH (2003) Natural selection for salt tolerance quantitative trait loci (QTLs) in wild sunflower hybrids: Implications for the origin of *Helianthus paradoxus*, a diploid hybrid species. Mol Ecol 12:1225–1235

Lexer C, Lai Z, Rieseberg LH (2004) Candidate gene polymorphisms associated with salt tolerance in wild sunflower hybrids: Implications for the origin of *Helianthus paradoxus*, a diploid hybrid species. New Phytol 161:225–233

Li Y, Ruperao P, Batley J, Edwards D, Khan T, Colmer TD, Pang J, Siddique KHM, Sutton T (2018) Investigating drought tolerance in chickpea using genome-wide association mapping and genomic selection based on whole-genome resequencing data. Front Plant Sci 9:190

Li W, Zhang H, Zeng Y, Xiang L, Lei Z, Huang Q, Li T, Shen F, Cheng Q (2020) A salt tolerance evaluation method for sunfower (*Helianthus annuus* L.) at the seed germination stage. Sci Rep 10:10626

Liang C, Wang W, Wang J, Liang C, Wang W, Wang J, Ma J, Li C, Zhou F, Zhang S, Yu Y, Zhang L, Li W, Huang X (2017) Identification of differentially expressed genes in sunflower (*Helianthus annuus*) leaves and roots under drought stress by RNA sequencing. Bot Stud 58:42

Lind EM, Borer E, Seabloom E, Adler P, Bakker JD, Blumenthal DM, Crawley M, Davies K, Firn J, Gruner DS et al (2013) Life-history constraints in grassland plant species: a growth-defence trade-off is the norm. Ecol Lett 16:513–521

Liu A, Burke JM (2006) Patterns of nucleotide diversity in wild and cultivated sunflower. Genetics 173:321–330

Livaja M, Unterseer S, Erath W, Lehermeier C, Wieseke R, Plieske J, Polley A, Luerßen H, Wieckhorst S, Mascher M, Hahn V, Ouzunova M, Schon CC, Ganal W (2016) Diversity analysis and genomic prediction of Sclerotinia resistance in sunflower using a new 25 K SNP genotyping array. Theor Appl Genet 129(2):317–329

Lofgren JR, Ramaraje Urs NV (1982). Chemically induced mutations in sunflower. In: Proceedings of the 10th International Sunflower Conference. Surfers Paradise, Australia. International Sunflower Association, Vlaardingen, Netherlands, 264–268

Loose LH, Heldwein AB, Lucas DDP, Hinnah FD, Bortoluzzi MP (2017) Sunflower emergence and initial growth in soil with water excess. Engenharia Agrícola 37(4):644–655

Mace ES, Singh V, Van Oosterom EJ, Hammer GL, Hunt CH, Jordan DR (2012) QTL for nodal root angle in sorghum (*Sorghum bicolor* L. Moench) co-locate with QTL for traits associated with drought adaptation. Theor Appl Genet 124:97–109

MacMillan K, Emrich K, Piepho HP, Mullins CE, Price AH (2006) Assessing the importance of genotype × environment interaction for root traits in rice using a mapping population II: conventional QTL analysis. Theor Appl Genet 113:953–964

Mahmoud AM, Ahmed TA (2016) Water use efficiency of sunflower genotypes under drip irrigation. Afr J Agric Res 11(11):925–929

Malhotra H, Vandana, Sharma S, Pandey R (2018) Phosphorus nutrition: plant growth in response to deficiency and excess. In: Hasanuzzaman M, Fujita M, Oku H, Nahar K, Hawrylak-Nowak B (eds) Plant nutrients and abiotic stress tolerance. Springe, Singapore, pp 171–190

Malidža G, Škorić D, Jocić S. (2000) Imidazolinone resistant sunflower (*Helianthus annuus* L.): inheritance of resistance and response towards selected sulfonyl urea herbicides. In: ISA, edition. Proceeding of the 15th International Sunflower Conference, vol 2. Toulouse, France, 12–15 June 2000, pp 42–47

Mandel JR, Dechaine JM, Marek LF, Burke JM (2011) Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, *Helianthus annuus* L. Theor Appl Genet 123:693–704

Mandel JR, Nambeesan S, Bowers JE et al (2013) Association mapping and the genomic consequences of selection in sunflower. Plos Genet 9:e1003378

Mangin B, Bonnafous F, Blanchet N, Boniface M-C, Bret-Mestries E, Carrère S et al (2017) Genomic prediction of sunflower hybrids oil content. Front Plant Sci 8:1633

Martin M, Molfetta P, Vannozzi GP, Zerbi G (1992) Mechanisms of drought resistance of *H. annuus* and *H. argophyllus*. In: Proceedings of the 13th international sunflower conference. Pisa, Italy, 7–11 Sept 1992. Intl. Sunflower Assoc., Paris. pp 571–586

Masalia RR, Temme AA, de leon Torralba N, Burke JM (2018) Multiple genomic regions influence root morphology and seedling growth in cultivated sunflower (*Helianthus annuus* L.) under well-watered and water-limited conditions. PLoS One 13(9):e0204279

Mehrabi Z, Pironon S, Kantar M, Ramankutty N, Rieseberg L (2019) Shifts in the abiotic and biotic environment of cultivated sunflower under future climate change. OCL 26:9

Meluca C, Pirvu N, Nistor T, Sturzi R, Stoilova A (2014) Sulfo technology for multiplication of sunflower hybrids resistant to tribenuron methyl-based herbicides. Agric Sci Technol 6(1):44–49

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

Mianlengeh ZE, Najafabadi MS, Saidi A, Askari H (2018) Monitoring response of a few bzip transcription factors in response to osmotic stress in sunflower. Iran J Biotechnol 16(2):e1422

Mickelbart M, Hasegawa P, Bailey-Serres J (2015) Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat Rev Genet 16:237–251

Miladinović D, Hladni N, Radanović A, Cvejić S, Jocić S (2019) Sunflower and climate change: possibilities of adaptation through breeding and genomic selection. In: Kole C (ed) Genomic designing of climate-smart oilseed crops. Springer International Publishing, Cham, pp 173–238

Miladinović D, Antunes D, Yildirim K, Bakhsh A, Cvejić S, Kondić-Špika A, Marjanovic Jeromela A, Opsahl-Sorteberg HG, Zambounis A, Hilioti Z (2021) Targeted plant improvement through genome editing: from laboratory to field. Plant Cell Rep. https://doi.org/10.1007/s00299-020-02655-4

Miller JF (1987) Sunflower, Vol: 2. In: Fehr W (ed) Principle of cultivar development. Macmillan Pub Co, NY, pp 626–668

Miller JF (1995) Inheritance of salt tolerance in sunflower. Helia 18:9–16

Miller JF, Al-Khatib K (2004) Registration of two oilseed sunflower genetic stocks, SURES-1 and SURES-2, resistant to tribenuron herbicide. Crop Sci 44:1037–1038

Miller JF, Seiler GJ (2003) Registration of five oilseed maintainer (HA 429-HA 433) sunflower germplasm lines. Crop Sci 43:2313–2314

Miller JF, Al-Khatib K (2000) Development of herbicide resistant germplasm in sun-flower. In: ISA, edition. Proceeding of the 15th international sunflower conference, vol 2. Toulouse, France, 12–15 June (2000) Intl. Sunflower Assoc., Paris, France, pp 419–423

Miller JF and Seiler GJ (2005) Tribenuron resistance in accessions of wild sunflower collected in Canada. In: Proceedings of 27th sunflower research workshop, Fargo, ND, 12–13 Jan 2005. Natl. Sunflower Assoc., Bismarck, ND, USA

Mitchell PJ, O'Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytol 197:862–872

Mithila J, Godar AS (2013) Understanding genetics of herbicide resistance in weeds: Implications for weed management. Adv Crop Sci Technol 1(4):115

Mustroph A (2018) Improving flooding tolerance of crop plants. Agronomy 8:160

Mykhailenko V, Kyrychenko V, Bragin A, Chuiko D (2019) Generation, evaluation, and prospects of further use of mutations based on new homozygous self-pollinated sunflower lines (Online First). IntechOpen. https://doi.org/10.5772/intechopen.89563

Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N, Özkan H, Chung G, Baloch FS (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Ozan Equip 32:261–285

Nagarathna TK, Shadakshari YG, Ramakrishna Parama, VR, Jagadish KS, Puttarangaswamy KT (2012) Examination of root characters, isotope discrimination, physiological and morphological traits and their relationship used to identify the drought tolerant sunflower (*Helianthus annuus* L.) genotypes. Helia 35(56):1–8. UDC 633.854.78:632.112(58.032.3). https://doi.org/10.2298/HEL1256001N

Nei M (1987) Molecular evolutionary genetics. New York, NY, USA: Columbia University Press. Genes (Basel). 2020 Mar. 11(3): 283. Published online 2020 Mar 6. https://doi.org/10.3390/genes11030283

Norton RDE, Roberts T (2015) Nitrogen use efficiency and nutrient performance indicators. Global Partnership on Nutrient Management Task Team Workshop, Washington DC, pp 1–14

Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. Mol Ecol 18(3):375–402

Olson B, Al-Khatib K, Aiken RM (2004) Distribution of resistance to imazamox and tribenuron-methyl in native sunflowers. In: Proceeding of the 26th sunflower res workshop. Fargo, ND, USA, 14–15 Jan 2004

Onemli F, Gucer T (2010) Response to drought of some wild species of *Helianthus* at seedling growth stage. Helia 33:45–54

Oosterhuis F, Papyrakis E, Boteler B (2014) Economic instruments and marine litter control. Ocean Coastal Manag 102:7–54

Ortiz R (2015) The importance of crop wild relatives, diversity, and genetic potential for adaptation to abiotic stress-prone environments. In: Redden R, Yadav SS, Maxted N, Dulloo ME, Guarino L, Smith P (eds) Crop wild relatives and climate change. Wiley, pp 80–87

Ortiz R, Crossa J, Franco J, Sevilla R, Burgueño J (2008) Classification of Peruvian highland maize races using plant traits. Genet Resour Crop Evol 55:151–162

Osorio J, Fernandez-Martinez JM, Mancha M, Garces R (1995) Mutant sunflower with high concentration in saturated fatty acid in the oil. Crop Sci 35:739–742

Ozturk Gokce ZN, Akbas S, Ayten S, Hussain Azimi M, Das R, Buse Guven S, Karabulut E, Omezli S, Uzer Z, Yerlikaya BA, Bakhsh A (2020) Abiotic stress tolerance in field crops: integration of omics approaches. In: Hasanuzzaman M (ed) Agronomic crops. Springer, Singapore, pp 503–526

Pace PF, Cralle HT, El-Halawany SHM, Cothren JT, Senseman S (1999) Drought-induced changes in shoot and root growth of young cotton plants. J Cotton Sci 3:183–187

Palmgren MG, Edenbrandt AK, Vedel SE, Andersen MM, Landes X, Osterberg JT, Falhof J, Olsen LI, Christensen SB, Sandoe P, Gamborg P, Kappel K, Thorsen BJ, Pagh P (2015) Are we ready for back-to-nature crop breeding? Trends Plant Sci 20(3):155–164

Paniego N, Eschaide M, Munoz M, Fernandez L, Torales S, Faccoi P, Fuxan I, Carrera M, Zandomeny R, Suarez E, Hopp H (2002) Microsatellite isolation and characterization in sunflower (*Helianthus annuus* L.) Genome 45:34–43

Panković D (1996) Photosynthesis in sunflower leaves. (*Helianthus annuus* L.) under water deficit conditions. Ph.D. Thesis, Faculty of Biology, University of Belgrade (In Serbian), pp 1–94

Parameswaran M (1996) Leaf angle as an indicator for gauging water stress in sunflowers. ISA Symposium II: Drought Tolerance in Sunflower. Beijing. PR China, June 14, 1996, pp 45–52

Pekcan V, Evci G, Yilmaz MI, Balkan Nalcaiyi AS, Çulha Erdal S, Cicek N, Arslan O, Ekmekci Y, Kaya Y (2016) Effects of drought stress on sunflower stems and roots. Intl J Adv Agric Environ Engg 3(1):96–102

Petcu E, Stanciu M, Stanciu D, Raducanu F (2008) Physiological traits for quantification of drought tolerance in sunflower. In: Velasco L (eds) Proceeding of the 17th international sunflower conferences, vol 1. Cordoba, Spain, 8–12 June 2008. Intl. Sunflower Assoc., Paris, France, pp 345–349

Pradhan C, Mohanty M (2013) Submergence stress: Responses and adaptations in crop plants. In: Rout GR, Das AB (eds) Molecular stress physiology of plants. Springer, India, pp 331–358

Prakash AH, Vajranabhaiah SN, Reddy PC (1993) Effect of salt stress on callus development from hypocotyl segments of sunflower (*Helianthus annuus* L.) genotypes. Helia 16(18):71–76

Prasad PVV, Staggenborg SA (2008) Impacts of drought and/or heat stress on physiological, developmental, growth and yield processes of crop plants. In: Ahuja LR, Reddy VR, Saseendran SA, Qiang Yu (eds) Response of crops to limited water: understanding and modelling water stress effects on plant growth processes, American society of agronomy. Crop Science Society of America, and Soil Science Society of America, Madison, pp 301–355

Presotto A, Ureta MS, Cantamutto M, Poverene M (2012) Effects of gene flow from IMI resistant sunflower crop to wild *Helianthus annuus* populations. Agric Ecosys Environ 146(1):153–161

Pustovoit VS (1967) Handbook of selection and seed growing of oil plants. Moscow, Russia: Kolos (In Russian, English translation from National Information Service. US Dept. of Commerce, Springfield, VA, USA)

Radanović A, Miladinović D, Cvejić S, Jocković M, Jocić S (2018) Sunflower genetics from ancestors to modern hybrids: a review. Genes 9(11):528

Ramu VS, Paramanantham A, Ramegowda V, Mohan-Raju B, Udayakumar M, Senthil-Kumar M (2016) Transcriptome analysis of sunflower genotypes with contrasting oxidative stress tolerance reveals individual and combined biotic and abiotic stress tolerance mechanisms. PLoS One 11(6):e0157522

Rauf S (2008) Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. Commun Biomet Crop Sci 3(1):29–44

Rauf S, Shahzad M, Teixeira da Silva JA, Noorka IR (2012) Biomass partitioning and genetic analyses of salinity tolerance in sunflower (*Helianthus annuus* L.). J Crop Sci Biotechnol 5:205–217

Rauf S, Sadaqat H, Ahmed R, Khan IA, (2009) Genetics of root characteristics in sunflower (*Helianthus annuus* L.) under contrasting water regimes. Indian J Plant Physiol 14(4):319–327

Razaq K, Rauf S, Shahzad M, Ashraf E, Shah F (2017) Genetic analysis of pollen viability: an indicator of heat stress in sunflower (*Helianthus annuus* L.). Intl J Innov Approach Agric Res 1(1):40–50

Razzaq H, Nadeem Tahir MH, Sadaqat AH, Bushra S (2017) Screening of sunflower (*Heliahus annuus* L.) accessions under drought stress conditions, an experimental assay. J Soil Sci Plant Nutr 17(3):662–671

Reif JC, Zhao Y, Würschum T, Gowda M, Hahn V (2013) Genomic prediction of sunflower hybrid performance. Plant Breed 132:107–114

Rogers CE, Thompson TE, Seiler GJ (1982) Sunflower species of the United States. National Sunflower Association, Bismarck, ND, USA, p 75

Ruggiero A, Punzo P, Landi S, Costa A, Van Oosten MJ, Grillo S (2017) Improving plant water use efficiency through molecular genetics. Horticulturae 3(2):31

Ruta N, Liedgens M, Fracheboud Y, Stamp P, Hund A (2010) QTLs for the elongation of axile and lateral roots of maize in response to low water potential. Theor Appl Genet 120:621–631

Sala CA, Bulos M, Echarte AM (2008a) Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. Crop Sci 48:1817–1822

Sala CA, Bulos M, Echarte AM, Whitt SR, Ascenzi R (2008b) Molecular and biochemical characterization of an induced mutation conferring imidazolinone resistance in sunflower. Theor Appl Genet 108:105–112

Sala CA, Bulos M, Altieri E, Weston B (2012a) Response to imazapyr and dominance relationships of two imidazolinone-tolerant alleles at the *Ahasl1* locus of sunflower. Theor Appl Genet 124:385–396

Sala CA, Bulos M, Alteri E, Ramos ML (2012b) Genetics and breeding of herbicide tolerance in sunflower. Helia 35(57):57–70

Sala CA, Bulos M, Altieri E, Ramos ML (2012c) Sunflower: improving crop productivity and abiotic stress tolerance. Wiley-VCH Verlag GmbH & Co. KGaA, pp 1205–1249

Sarazin V, Duclercq J, Guillot X, Sangwan B, Sangwan RS (2017) Water-stressed sunflower transcriptome analysis revealed important molecular markers involved in drought stress response and tolerance. Envir Exp Bot 142:45–53

Sarpe N, Poienaru S, Maschio M (2007) No-tillage system applied to the sunflower (hybrid pioneer PR64E83) resistant to the tribenuron-methyl in the conditions from Romania. Commun Agric Appl Biol Sci 72(2):1–7

Sato NE, Pereyra-Irujo G, Creus CM, Aguirrezabal AN (2012) Correlation between the response of leaf expansion and hypocotyl elongation to water deficit in sunflower (*Helianthus annuus* L.) genotypes. In: Proceedings of the 18th international sunflower conference, Mar del Plata & Balcarce, Argentina, 27 Feb-Mar 1, 2012. Intl. Sunflower Assoc., Paris, France, p 127

Savin VN, Stepanenko OG (1968) Action of gamma rays from 60Co on sunflower. Agric Biol 3:921–922

Schuster W, Kubler I (1983) Possibilities of increasing the genetic variability due to seed quality composition. Helia 6:5–12

Scoffoni C, Albuquerque C, Brodersen CR, Townes SV, John GP, Cochard H, Buckley TN, McElrone AJ, Sack L (2017a) Leaf vein xylem conduit diameter influences susceptibility to embolism and hydraulic decline. New Phytol 213:1076–1092

Scoffoni C, Sack L, Ort D (2017b) The causes and consequences of leaf hydraulic decline with dehydration. J Exp Bot 68:4479–4496

Seiler GJ (2007) Wild annual *Helianthus anomalus* and *H. deserticola* for improving oil content and quality in sunflower. Ind Crop Prod 25:95–100

Seiler GJ, Gulya TJ, Marek L (2006) Exploration for wild *Helianthus* species from the desert southwestern USA for potential drought tolerance. Helia 29:1–10

Seiler GJ, Qi LL, Marek LF (2017) Utilization of sunflower crop wild relatives for cultivated sunflower improvement. Crop Sci 57:1–19

Seiler GJ (2012) Utilization of wild *Helianthus* species in sunflower breeding. In: Kovačević Z, Škorić D, Sakač Z (eds) Sunflower genetics and breeding. Serbian Academy of Science, Serbia, pp 355–413

Seiler GJ (2018) Value of sunflower crop wild relatives' habitat diversity for sunflower in a changing world. In: Proceedings of international symposium sunflower and climate change. Toulouse France, 5–6 Feb 2018

Seiler G, Jan CC (2010) Basic information. In: Hu J, Seiler G, Kole C (eds) Genetics, genomics and breeding of sunflower. Science Publishers, Enfield, New Hampshire, USA, CRC Press, Boca Raton, FL, pp 1–40

Shaik R, Ramakrishna W (2013) Genes and co-expression modules common to drought and bacterial stress responses in Arabidopsis and rice. PLoS ONE 8:e77261. https://doi.org/10.1371/journal.pone.0077261

Shu-tian L, Yu D, Tian-wen G, Ping-liang Z, Ping H, Majumdar K (2018) Sunflower response to potassium fertilization and nutrient requirement estimation. J Integr Agric 17(12):2802–2812

Singh BD (2000) Plant breeding—principles and methods. Kalyani Publishers. Ludhiana, New Delhi, pp 1–896

Singh BD (2004a) Textbook of plant breeding. Kalyani Publishers, New Delhi, pp 123–125

Singh AK (2004b) The physiology of salt tolerance in four genotypes of chickpea during germination. J Agric Sci Technol 6:87–93

Škorić D (1989) Sunflower breeding. In: Polak V (ed) Sunflower-monograph. Nolit, Beograd, pp 284–393 (In Serbian)

Škorić D (1992) Achievements and future directions of sunflower breeding. Field Crops Res 30:231–270

Škorić D (2009) Sunflower breeding for resistance to abiotic stresses. Helia 32:1–16

Škorić D (2016) Sunflower breeding for resistance to abiotic and biotic stresses. In: Shanker AK, Shanker C (eds) Abiotic and biotic stress in plants—recent advances and future perspectives. Intech, London, pp 585–635

Škorić D (2012) Sunflower breeding. In: Škorić D, Sakač Z (eds) Sunflower genetics and breeding. (International Monography). Serbian Academy of Sciences (SA- SA), Branch in Novi Sad, Novi Sad, Republic of Serbia, pp 164–344

Soldatov KI (1976) Chemical mutagenesis in sunflower breeding. In: Proceeding of the 7th international sunflower conference. Krasnodar, USSR. 27 June–3 July 1976. Intl. Sunflower Assoc., Vlaardingen, the Netherlands, pp 352–357

Song J, Wang B (2015) Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model. Ann Bot 115:541–553s

Song Y, Kirkham MB, Ham JM, Kluitenberg GJ (1999) Dual probe heat pulse technique for measuring soil water content and sunflower water uptake. Soil Tillage Res 50:345–348

Suresha PG, Kulkarni VV, Supriya SM, Darshan S, Patil CB (2017) Genetic diversity analysis in sunflower (*Helianthus annuus* L.) parental lines using SSR and RAPD markers. Int J Curr Microbiol App Sci 6(7):2069–2076

Tamang BG, Fukao T (2015) Plant adaptation to multiple stresses during submergence and following de-submergence. Intl J Mol Sci 16:30164–30180

Tan AS, Kaya J (2019) Sunflower (*Helianthus annuus* L.) genetic resources, production and researches in Turkey. OCL 26:1–7

Tan S, Evans RR, Dahmer M, Singh BK, Shaner DL (2005) Imidazolinone tolerant crops: history, current status and future. Pest Manag Sci 61:246–257

Tang S, Knapp SJ (2003) Microsatellites uncover extraordinary diversity in native American landraces and wild populations of cultivated sunflower. Theor Appl Genet 106:990–1003

Tardieu F (2012) Any trait or traitrelated allele can confer drought tolerance: just design the right drought scenario. J Exp Bot 63:25–31

Tardieu F (2013) Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. Front Physiol 4(17):17

Tavoljansky NP, Chiryaev PV, Scherstyuk SV, Altdnnikova VL (2004) Development of original material for sunflower breeding for seed characteristics, oil and protein quality in the conditions of central Chernozem region. Helia 27:117–122

Terzić S, Zorić M, Seiler G (2019) Qualitative traits in sunflower breeding: UGA-SAM1 phenotyping case study. Crop Sci 60:303–319

Terzić S, Marie-Claude Boniface MC, Marek L, Alvarez D, Baumann K, Gavrilova V, Joita-Pacureanu M, Sujatha M, Valkova D, Velasco L, Hulke BS, Jocić S, Langlade N, Muños S, Rieseberg L, Seiler G, Vear F (2020) Gene banks for wild and cultivated sunflower genetic resource. OCL 27:9

Terzić S, Miklič V, Čanak P (2017) Review of 40 years of research carried out in Serbia on sunflower pollination. OCL 24:(6)D608

Tetreault HN, Kawakami T, Ungerer MC, Levy C (2016) Low temperature tolerance in the perennial sunflower *Helianthus maximiliani.* Amer Midl Nat 175:91–102

Tishchenko OM, Komisarenko AG, Mykhalska SI, Sergeeva LE, Adamenko NI, Morgun BV, Kochetov AV (2014) Agrobacterium-mediated transformation of sunflower (*Helianthus annuus* L.) in vitro and in planta using LBA4404 strain harbouring binary vector pBi2E with dsRNA-suppressor of proline dehydrogenase gene. Cytol Genet 48:218–226

Todesco M, Owens GL, Bercovich N, Légaré JS (2020) Large chromosomal variants drive adaptation in sunflowers. Nat Plants 6(7):734–735

Torres AM, Diedenhofen U (1981) Sunflower alcohol-dehydrogenase genotypes—germination rates and response to flooding. Environ Exp Bot 21:35–44

Tyagi V, Dhillon SK, Kaushik P, Kaur G (2018) Characterization for drought tolerance and physiological efficiency in novel cytoplasmic male sterile sources of sunflower (*Helianthus annuus* L.). Agronomy 8:232

Uga Y, Okuno K, Yano M (2011) *Dro1*, a major QTL involved in deep rooting of rice under upland field conditions. J Exp Bot 62:2485–2494

Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, 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

UPOV (2000). Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability: Sunflower (Helianthus annuus L.). TG/81/6. Geneva, Switzerland: International Union for the Protection of New Varieties of Plants. Available from: http://www.upov.int/edocs/tgdocs/en/tg081.pdf (Accessed on 7 Sep 2017)

Umar M, Siddiqui ZS (2018) Physiological performance of sunflower genotype under combined salt and drought stress environment. Acta Bot Croat 77(1):36–44

Van Dongen JT, Licausi F (2015) Oxygen sensing and signalling. Annu Rev Plant Biol 66:345–367

Varotto S, Tani E, Abraham E, Krugman T, Kapazoglou A, Melzer R, Radanović A, Miladinović D (2020) Epigenetics: possible applications in climate-smart crop breeding. J Exp Bot 71:5223–5236

Vassilevska-Ivanova R, Shtereva L, Kraptchev B, Karceva T (2014) Response of sunflower (*Helianthus annuus* L) genotypes to PEG-mediated water stress. Centr Eur J Biol 9(12):1206–1214

Velasco L, Perez-Vich B, Fernandez-Martinez JM (1999) The role of mutagenesis in the modification of the fatty acid profile of oilseed crops. J Appl Genet 40(3):185–209

Voesenek LACJ, Bailey-Serres J (2015) Flood adaptive traits and processes: an overview. New Phytol 206:57–67

Vranceanu AV (2000) Floarea-soarelui hibrida. Editura Ceres, Bucuresti. (In Romanian), pp 1–1147

Vrânceanu AV, Craiciu DS, Soare G, Păcureanu-Joiţa M, Voinescu G, Sandu I (1992) Sunflower genetic resistance to Phomopsis attack. In: ISA, edition. Proc. of the 13th Int. Sunf. Conf. Pisa. Italy. September 7–11. 1992. Intl. Sunflower Assoc. Paris. France, 2:1301–1306

Vrbničanin S, Božic D, Malidža G, Dušanič N, Pavlovič D, Barac M (2008) Tolerance of sunflower (*Helianthus annuus* L.) to imazethapyr. Helia 31:85–94

Wample RL, Reid DM (1978) Control of adventitious root production and hypocotyl hypertrophy of sunflower (*Helianthus annuus*) in response to flooding. Physiol Planta 44:351–358

Wang N, Yang C, Pan Z, Liu Y, Peng S (2015) Boron deficiency in woody plants: various responses and tolerance mechanisms. Front Plant Sci 6:916

Warburton ML, Rauf S, Marek L, Hussain M, Ogunola O, de Jesus Sanchez Gonzalez J (2017) The use of crop wild relatives in maize and sunflower breeding. Crop Sci 57:1227–1240

Watanabe M, Shinmachi F, Noguchi A, Hasegawa I (2005) Introduction of yeast Metallothionein gene (*CUP1*) into plant and evaluation of heavy metal tolerance of transgenic plant at the callus stage. Soil Sci 51:129–133

Weih M, Hamnér K, Pourazari F (2018) Analyzing plant nutrient uptake and utilization efficiencies: comparison between crops and approaches. Plant Soil 430:7–21

Welch ME, Rieseberg LH (2002) Habitat divergence between a homoploid hybrid sunflower species, *Helianthus paradoxus* (Asteraceae), and its progenitors. Amer J Bot 89:472–478

Wenhui L, Zhang H, Zeng Y, Xiang L, Lei Z, Huang Q, Li T, Shen F, Cheng Q (2020) A salt tolerance evaluation method for sunflower (*Helianthus annuus* L.) at the seed germination stage. Sci Rep 10:10626

Weston B, McNevin G, Carlson D (2012) Clearfield® plus technology in sunflowers. In: Proceeding of the 18th international sunflower conference. Mar del Plata, Argentina, pp 149–154

Wu CI (2001) The genic view of the process of speciation. J Evolut Biol 14(6):851–865

Würschum T, Reif JC, Kraft T, Janssen G, Zhao Y (2013) Genomic selection in sugar beet breeding populations. BMC Genet14:85. https://doi.org/10.1186/1471-2156-14-85

Xiao G, Zhang Q, Xiong Y, Lin M, Wang J (2007) Integrating rainwater harvesting with supplemental irrigation into rain-fed spring wheat farming. Soil till Res 93:429–437

Xu Z, Marowa P, Han Liu H, Du H, Zhang CS (2020) Genome-wide identification and analysis of P-type plasma membrane H+-ATPase sub-gene family in sunflower and the role of *HHA4* and *HHA11* in the development of salt stress resistance. Genes. https://doi.org/10.3390/genes11040361

Yasumoto S, Terakado Y, Matsuzaki M, Okada K (2011) Effects of high-water table and short-term flooding on growth, yield, and seed quality of sunflower. Plant Prod Sci 14(3):233–248

Ynet N, Yilancioglu K (2018) A CRISPR/Cas9 model of sunflower (*Helianthus annuus* L.) resistance for biotic and abiotic stresses. New Biotechnol 44:S84

Zambelli A, León A (2015) Mutagenesis in Sunflower. In: MartÍnez Force E, Dunford NT, Salas JJ (eds) Sunflower oilseed. Chemistry, production, processing and utilization. JJAOCS monograph series on oilseeds, vol 7. Chapter: 2, pp 27–52

Zhang Z (2016) Use of genetic transformation technology in oil crops: soybean and sunflower. Dissertation, Ohio State University

Chapter 4
Integration of Genomics Approaches in Abiotic Stress Tolerance in Groundnut (*Arachis hypogaea* L.): An Overview

B. Aravind, Spurthi N. Nayak, Rakeshkumar S. Choudhary, Spoorti S. Gandhadmath, P. V. V. Prasad, Manish K. Pandey, Ramesh S. Bhat, Naveen Puppala, Putta Latha, Palagiri Sudhakar, and Rajeev K. Varshney

Abstract In recent years, the effect of climate change on agriculture is perceived with irregular rainfall trends, temperature patterns and disease/pest outbreaks. Productivity of many crop species is impaired by abiotic stress such as heat, salinity, drought, cold and heavy metal stresses. In groundnut, the impact of high temperatures and drought on crop growth and development have been extensively studied. In order to meet global food and nutritional welfare, there is a need to consider the physiological responses and molecular pathways underlying abiotic stress tolerance. Several traits like water use efficiency (WUE), chlorophyll content, photosynthetic rate, stomatal conductance, root traits and yield-related parameters were found to be affected by abiotic stresses in groundnut. The availability of germplasm resources and genomic technologies help in exploring the tolerant genotypes conferring abiotic stress tolerance. The genomic regions associated with the tolerance related traits were studied using genetic linkage and association-based approaches using different types of molecular markers in groundnut. Latest advances in sequencing and artificial intelligence-based gene prediction have improved the process of identifying possible genes that impart abiotic stress tolerance. The transcriptomics, proteomics, metabolomics and genetic engineering approaches were utilized for imparting drought tolerance. Though genomics studies related to heat,

---

B. Aravind · S. N. Nayak (✉) · R. S. Choudhary · S. S. Gandhadmath · R. S. Bhat
Department of Biotechnology, University of Agricultural Sciences, Dharwad, India
e-mail: nayaksn@uasd.in

P. V. V. Prasad
Department of Agronomy, Kansas State University, Manhattan, USA

M. K. Pandey · R. K. Varshney
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India

N. Puppala
Agricultural Science Center at Clovis, New Mexico State University, Clovis, NM, USA

P. Latha · P. Sudhakar
Regional Agricultural Research Station (RARS), Acharya N G Ranga Agricultural University (ANGRAU), Tirupati, India

C. Kole (ed.), *Genomic Designing for Abiotic Stress Resistant Oilseed Crops*,
https://doi.org/10.1007/978-3-030-90044-1_4

salinity, cold tolerance are very limited in groundnut, there is high potential to utilize available resources and multi-omics technologies to improve groundnut for abiotic stress tolerance.

Keywords Peanut · Drought · Heat · Salinity · Multi-omics · Transgenics · Marker-trait association

4.1 Introduction

Climate change and climate variability affect the growth, development and productivity of crop plants. Climate change influences biotic and abiotic stresses in cultivated crops adversely affecting the productivity and quality of the produce. Climate changes cause erratic rainfall patterns, drought, high temperature, chilling, flooding, salinity, increased carbon dioxide concentration and other greenhouse gases. Besides, these changes influence the occurrence and intensity of new pests, diseases and their sporadic spread. Thus, climate change aggravates both biotic and abiotic stresses in cultivated crops thereby negatively influencing the productivity of several crop species including groundnut which could intensify the concerns for malnutrition and poverty across the globe (Hatfield and Prueger 2015; Kole et al. 2015).

Groundnut or peanut is a self-pollinated annual legume largely cultivated in dry areas of tropics and subtropics. It belongs to the family Fabaceae and its botanical name is *Arachis hypogaea* Linn., a Greek-derived word referred to the plant that produces fruits or pods under the ground or soil. It is one of the world's principal economic crops and is the second most important annual oilseed crop after soybean (*Glycine max* L. Merr). It is believed to be a native of Brazil (South America).

Groundnut is primarily valued because of its richness in edible oil and vegetable proteins. It is rightly called as 'king of oilseeds' because of its contribution towards edible oil industries. Groundnuts contain about 47–53% oil; 25–36% protein; 10–15% carbohydrate and are rich sources of phosphorus, vitamins B and E. The groundnut crop can be grown from 40° N to 40° S of the equator. It is cultivated in about 29.59 million hectares' area in the world with production of 48.75 million tons. China leads in groundnut production by 36% globally followed by India, Nigeria and the United States of America (FAOSTAT 2019).

The exposure to certain biotic and abiotic stresses is the major constraint, which hinder groundnut productivity. Biotic stresses include the incidence of diseases like rust, stem rot, early and late leaf spot, *Aspergillus flavus*, groundnut bud necrosis disease, bacterial wilt and the insect pest like tobacco cutworm, gram pod borer, jassids, thrips, etc., while abiotic stresses primarily include the incidence of drought, high-temperature, low-temperature, salinity, phosphorus deficiency and calcium-induced iron chlorosis. Drought and high temperature or the combination of both are major abiotic constraints in groundnut growing regions. Drought occurring in mid and at the end of growing season severely damage the crop by affecting pod yield. Terminal drought in *Valencia* groundnut genotypes revealed that the drought stress reduced biomass production at harvest by 13.03%, pod yield by 33.16% and the number of mature pods per plant by 36.54% (Carvalho et al. 2017). The reduction in the number of mature pods per plant was the main cause of yield loss (Carvalho et al. 2017). Hamidou et al. (2012) reported a 72% decrease in the pod yield due to drought stress at high temperatures and a 55% decrease at moderate temperatures. The harvest index (HI) was decreased by 50% during the hotter season and by 25% during the moderately hot season. The duration and intensity of drought and the growth stages at which drought occurs have an impact on groundnut yields (Awal and Ikeda 2002). Drought stress boost the incidence of *Aspergillus flavus* and aflatoxin contamination (Craufurd et al. 2006). There were significant negative linear relations between infection and fraction of extractable soil water between flowering and harvest. The study stated that the infection severity and concentration of aflatoxin in groundnut can be correlated with the incidence of drought stress during pod-filling when soil temperatures are ideal for the growth of *Aspergillus flavus* (Craufurd et al. 2006).

The ambient temperature for growth of groundnut is between 25 and 30 °C and for flowering and maturity is about 28–33 °C. The increase in mean air temperature of 23 °C is predicted to mitigate the groundnut yield by 23–36% in India (Hundal and Kaur 1996). The day temperature more than 35 °C during the reproductive phase reduces the fruit set and pod yield (Prasad et al. 1999a) that was due to fewer pollen grains and reduced pollen viability (Prasad et al. 1999b, 2000a). Higher soil temperature (38/22 °C; daytime maximum/night-time minimum) affected the flower, peg and nodule formation in groudnut (Prasad et al. 2000b, 2001). Temperature below 18–20 °C results in delay in germination in groundnut resulting in poor growth of plants with delayed maturity (Bhagat et al. 1992). Salinity is another major abiotic stress that reduces the plant's ability to absorb water, causes ionic imbalance, and generates reactive oxygen species (ROS) (Azad et al. 2014). In calcareous black clay soil application of phosphorus in groundnut has shown a significant response in grain yield and yield components (Singh and Singh 2000; Kamara et al. 2011).

Groundnut being a C_3 plant showed increase in growth and biomass production under elevated CO_2 conditions as compared to ambient CO_2 conditions. Climate change in terms of elevated CO_2 alone or combination with optimal temperature has favored groundnut growth, development and yield (Shwetha et al. 2017). Elevated CO_2 (700 μmol mol^{-1}) increased leaf photosynthesis across all temperatures in the range for 32/22 °C to 44/34 °C (Prasad et al. 2003). However, super-optimal temperatures were detrimental to groundnut reproductive processes (pollen viability, seed set, individual seed weight and seed HI) under both optimal and elevated levels of CO_2 (Prasad et al. 2003).

Considering the food value and economic value, groundnut is important in the present era of changing climate and overcoming poverty and malnutrition in developing countries. There is scope to enhance the beneficial properties of groundnut by using potential genotypes from available genetic resources and wild relatives using plant breeding approaches. However, conventional or traditional breeding approaches have limitations. For example, the development of drought-tolerant crops through traditional breeding is time-intensive and laborious because of quantitative nature of drought tolerance (Ribaut et al. 1997). The incorporation of resistance genes from distant relatives of *Arachis* species is difficult due genetic incompatibility. It is important to include new omics technologies along with the traditional breeding approaches to improve groundnut genotypes that are climate resilient (Kambiranda et al. 2011).

4.2 Description of Different Abiotic Stresses

The external pressure or environmental conditions that negatively impacts the plant growth and maturity are stress responses (Levitt 1972, 1980). The effects of stresses are measured in terms of plant survival, crop yield, growth (biomass) and primary assimilatory processes (carbon assimilation, mineral uptake).

The plant's ability to cope up with the adverse environment is associated with stress tolerance. Plants overcome the stress either by avoidance, where plants respond by reducing the impact of the environmental stresses or by tolerance, where the plant's genetic/ physiological properties help to withstand the stress conditions. In groundnut, the yield losses are mainly attributed to abiotic stresses viz., drought, high temperature, salinity and heavy metal stresses. The impact of abiotic stress on the growth and development of the groundnut crop are presented in Fig. 4.1.

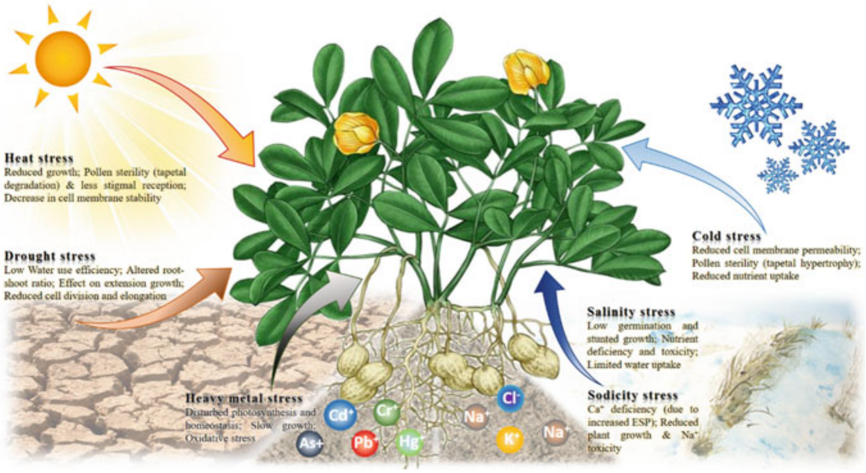

Fig. 4.1 Effect of abiotic stress on groundnut (*Arachis hypogaea*)

4.2.1 Drought Tolerance

Severe moisture stress during crop growth adversely affect the pod yield, quality of seeds and higher aflatoxin contamination in groundnut (Holbrook and Stalker 2010). Terminal drought conditions in groundnut resulted in 24% yield reduction as compared to moisture stress at vegetative phases. Hence it is observed that the duration of drought and its affect on reproductive phase has influenced on yield reduction (Boontang et al. 2010). Several plant characteristics like root traits, leaf traits and WUE are important for increasing the tolerance to abiotic stresses.

Breeding for drought tolerance basically depends on the yield advantage of tolerant genotypes that can extract water from the soil under moisture stress conditions. In this regards, the root architecture (root length, rooting depth and root dispersion) plays an integral role in the absorption of water and nutrient (Matsui and Singh 2003; Reddy et al. 2003; Nigam et al. 2005) and genotypes with such traits produced relatively higher yield even under prolonged drought conditions in the vegetative stages (Songsri et al. 2008, 2009). Under terminal drought conditions, leaves play important role by reducing photosynthetic activity by inculcating high stomatal resistance whereas roots do not respond during the late growth stages (Jongrungklang et al. 2014). Increased WUE is found to be the result of the association between decreased net photosynthetic rate and stomatal closure (Ruggiero et al. 2017). However, higher biomass production and harvest index was observed (Nigam et al. 2005; Ratnakumar and Vadez 2011; Koolachart et al. 2013). Generally early-maturing genotypes yield more than the late-maturing genotypes under drought conditions. However, early maturing groundnuts if exposed to terminal drought conditions, there is a reduction in the yield (Kashiwagi et al. 2006). The traits like specific leaf area (SLA), transpiration rate, chlorophyll content, relative water content and WUE are important traits that are considered in drought tolerance studies in groundnut.

“WUE is broadly defined as the ratio of water used by the plant for metabolism to the water lost through transpiration and is an indicator of selecting the crops for drought tolerance” (Udayakumar et al. 1998; Evans and Sadler 2008; Rao et al. 1995; 2001). The drought tolerance in groundnut was studied by screening several of these physiological traits (Table 4.1).

Apart from the phenotypical and physiological effects experienced by the plant under drought stress, it is observed that aflatoxin contamination is associated with the drought. Hence usage of drought-tolerant germplasm resistant to aflatoxin has become a prominent trait for selection (Guo et al. 2008; Fountain et al. 2014). It is also observed that there was no significant effect of aflatoxin contamination over reduction in yield under drought condition that suggests that drought tolerant mechanism and the disease incidence might be independent (Hamidou et al. 2014).

Table 4.1 Screening for abiotic stress tolerance in groundnut

Traits screened	No. of accessions screened	No. of accessions selected	Selection base	References
Drought tolerance				
SCMR, SLA, vegetative, reproductive and quality traits	184 and 4 cultivars	18	High SCMR and low SLA	Upadhyaya (2005)
SLA, SCMR, root dry weight, harvest index and WUE	11	2	High WUE and large root system	Songsri et al. (2009)
CO_2 assimilation rate, stomatal conductance, transpiration efficiency, SLA, SPAD, stomata number per square centimetre and per leaf and canopy temperature	18	2	High yielding in VC region	Balota et al. (2012)
41 morpho-agronomic traits	269 (comprises 20 *Arachis* sps.)	20	Superior agronomic, nutritional quality and drought related traits	Upadhyaya et al. (2011)
Leaf morphology, transpiration profile, SPAD chlorophyll meter readings (SCMR), SLA and transpiration rate per leaf area)	2 (*Arachisduranensis* and *Arachisipaensis*)	–		Leal-Bertioli et al. (2012)
Under intermittent drought and fully irrigated conditions	268	3 (ICG 5891, ICG 6057, ICG 9777)	High yielding genotypes under drought	Hamidou et al. (2013)
Transpiration (T), transpiration efficiency (TE), SLA and SPAD chlorophyll meter reading (SCMR)	2 RIL populations	-	Drought and yield related traits	Gautami et al. (2012a)

(continued)

Table 4.1 (continued)

Traits screened	No. of accessions screened	No. of accessions selected	Selection base	References
Heat tolerance				
SLA and relative leaf injury (RI)	38 accessions of 12 wild *Arachis* species	A. *glabrata* 11824	Heat tolerance	Nautiyal et al. (2008)
Temperature induction response (TIR)	20	4 (Heat tolerant) 6 (moderately tolerant)	TIR	Rani et al. (2018)
Partitioning coefficient (p)	–	–	Heat tolerance in groundnut was evaluated under field conditions using physiological traits	Ntare et al. (2001)
Intermittent water condition and fully irrigated condition, partition rate	268	–	Proportion of dry matter partitioned into pods was (partition rate)	Hamidou et al. (2013)
Soil temperature, air temperature and cellular membrane thermostability	–	6	Tolerance to high air and soil temperature in controlled environments	Craufurd et al. (2003)
Cold tolerance				
Low temperature at germination	1704	–	The accessions which showed 80% or more germination were considered as low temperature tolerance at germination	Upadhyaya et al. (2001)

(continued)

Table 4.1 (continued)

Traits screened	No. of accessions screened	No. of accessions selected	Selection base	References
15 morphological traits and 15 agronomic traits	158 cold temperature. tolerant cultivars with control	158	The cold tolerance accessions were superior to control cultivars for agronomic trait compared with their respective controls	Upadhyaya et al. (2009)
SLA and relative leaf injury (RI)	38 accessions of 12 wild *Arachis* species	*A. paraguariensis* 12042	Cold tolerant	Nautiyal et al. (2008)
Salinity tolerance				
Plant mortality and seed yield	127	11 (salinity tolerant) & 10 (moderately tolerant)	High plant stand and > 50 gm^{-2} seed yield—salinity tolerant & yield > 35 gm^{-2}—moderately tolerant	Singh et al. (2008)
Plant mortality, seed yield and nutrient absorption	210	10 (salinity tolerant) & 21 (moderately tolerant)	High plant stand and > 150 gm^{-2} seed yield—salinity tolerant & yield > 100–149 gm^{-2}—moderately tolerant	Singh et al. (2016)
Yield characteristics	275	14 (Tolerant) ICGV 87187, ICGS 76 most tolerant	Pod-seed yield and pod-seed number	Srivastava et al. (2018)
Mineral/metal stress				
Response of N-fixation	17	1 (ICGV 86015)	Tolerant under soil drying	Devi et al. (2010)
Reaction to Al toxicity	8	2	Root volume and dry weight	Liaoet al. (2000)
SCMR, SLA, RWC, CSI, MSI, Gas exchange parameters, Root traits	274	8	Drought and Yield related traits	Sudhakar et al. (2013)

4.2.2 Heat Tolerance

The physiological responses of heat (high temperature) stress impacts both water and nutrient absorption and uptake due to reduced activities of nutrient-metabolizing enzymes like nitrate reductase (Huang et al. 2012; Klimešová et al. 2020). “The reproductive processes involving pollen and stigma viability, pollination, anthesis, pollen tube growth, and early embryo development are particularly vulnerable to heat stress”(Giorno et al. 2013; Hamidou et al. 2013). However, pollen is more sensitive to heat than stigma (Lamaoui et al. 2018).

Like in other crops, the groundnut productivity is also affected by high-temperature stress especially during the reproductive stages of the crop. The hot days and warm nights limit the groundnut pod set due to reduced pollen viability and pollen count. Heat stress has a greater negative effect on flowering and microsporogenesis (Prasad et al. 2000a; Craufurd et al. 2003). Studies on the solute leakage and chlorophyll fluorescence parameters in leaves at the time of heat stress reduced chlorophyll content (Chauhan and Senboku 1997; Nautiyal et al. 2008). The differences in chlorophyll fluorescence and membrane thermostability were also found to be good indicators for the high-temperature tolerance (Talwar et al. 1999).

The light-dependent chemical reactions and carbohydrate metabolism occurring in chloroplasts are adversely affected due to heat stress resulting in an effect on the photosynthetic rate (Prasad et al. 2015). Besides, the membrane function and membrane integrity plays an integral role over photosynthesis and respiration rates. The membrane stability during the stress plays an important role in exhibiting high-temperature tolerance. The estimation of the malondialdehyde (MDA), obtained with lipid peroxidation is used in screening for heat tolerance. The heat stress also induces heat shock proteins (HSPs) in plants. These are a unique set of low molecular mass proteins with various molecular sizes like HSP17, HSP40, HSP60, HSP70, HSP100. They help the cells to endure the heat stress by acting as molecular chaperones protecting essential enzymes and nucleic acids from denaturation and misfolding due to high temperature (Jain 2000).

All the plants under continuous heat stress tend to produce ROS and its accumulation causes membrane polarization at the plasma membrane outer surface and activates the RBOHD (Respiratory burst oxidase homolg protein-D), a ROS-producing enzyme located at the plasma membrane. The ROS accumulation may lead to programmed cell death (PCD) (Qi et al. 2011) and acts as signal to trigger heat shock response in the plants (Asada 2006).

Drought and heat stresses often coincide and have varied impact on the plant’s growth and development. The combined impact of drought and heat have a more detrimental effect on plant growth than independently. The heat stress influences membrane fluidity by affecting the integrity of protein and lipids in the membrane and induces membrane leakiness. The thylakoid membranes of chloroplast are highly prone to heat and drought stress that affect photosynthetic rate and act as primary indicators (Prasad et al. 2008). The screening of several physiological traits to identify the genotypes with high-temperature stress tolerance in groundnut are indicated in Table 4.1.

4.2.3 Salinity Tolerance

Salinity, another important crop productivity limiting abiotic stress that reduces the plants' ability to absorb water besides affecting ionic balance, chloroplast stromal value and ROS production (Azad et al. 2014). Increasing soil salinity decreased haulm weight and pod yield by 47.7% and 53.6%, respectively, while seed oil content and protein content are decreased by 7.84% and 12.1%, respectively (Azad et al. 2014). An increase in soil salinity influences the nutrient status of the plant. The rise in soil salinity levels from 6.3 to 11.1 dSm^{-1} resulted in decrease in nitrogen and phosphorous content of haulm, while potassium content remained unaffected (El-Rheemkh and Zaki 2015). Meena et al. (2017) reported that the salinity level in water up to 2.0 dSm^{-1} and root zone soil salinity of 3.21 dSm^{-1} has no significant effect over groundnut pod and haulm yield under calcareous black clay soil conditions. A study on the effect of salinity on two groundnut varieties (Dacca-1 and Zhinga) showed a significant decrease in germination percentage with an increase in salt concentration. It also caused a sharp reduction in chlorophyll a, b and total chlorophyll content in both genotypes (Akter et al. 2020). The total number of pods per plant was reduced under salinity and pod weight showed significant variation under saline conditions which proved to be the best trait for salinity tolerance screening (Srivastava et al. 2018). Apart from these, a negative trend was observed in traits like, seedling emergence, radicle elongation, plant height and dry matter weight with increase in salinity (Mensah et al. 2006).

"Soil sodicity is the accumulation of sodium salt relative to other types of salt cations like calcium and magnesium, which is caused by increased soil pH." Effects of exchangeable sodium percentage in groundnut showed severe effects of sodicity with results depicting about 50% loss of yield, a continuous decrease in dry matter and detoriation in protein content and oil quality (Singh et al. 1985). An increase in exchangeable sodium percentage has significantly reduced the availability of nutrients like K, Ca and N which makes groundnut a sensitive crop towards sodicity. Iron chlorosis is often observed in soils with higher pH and calcareous soils, limiting the crop productivity (Prasad et al. 2000c).

Genotypes differ in their response to iron chlorosis and foliar application of ferrous sulphate can help to manage iron deficiency in groundnut (Prasad et al. 2000c). The flooding and submergence situations are rare in groundnut cultivated areas and no published reports indicate the tolerance to submergence/flooding in groundnuts.

4.2.4 Cold Tolerance

The sub optimal temperatures can affect the plant's metabolism leading to two types of injuries viz., chilling and freezing injury. Chilling injury is when a plant gets injured above freezing temperatures (0–15 °C) and the latter is when the plant is exposed to freezing temperatures (<0 °C) leading to injury. Plants differ in their tolerance to chilling and freezing temperatures (Wani et al. 2016; Jain 2000). Acclimatization for cold tolerance is attained when a plant is exposed to chilling, yet non-freezing temperatures gradually let the plants to adapt to the cold environments.

Low temperatures are a limiting factor for groundnuts' crop development and can be a potential reason for yield loss. Cold temperatures have been shown to affect germination, dry matter accumulation, shelling percentage and yield (Table 4.1). About 158 groundnut accessions that were tolerant to low temperature (12 °C) at germination stage showcased superiotity in several agronomic traits against control across seasons. They can serve as breeding material for genetically diverse cold-tolerant high-yielding groundnut cultivars (Upadhyaya et al. 2009).

4.2.5 Heavy Metal Stress Tolerance

Most of the heavy metals like mercury, cadmium, chromium, lead and arsenate have shown to affect the plant growth (especially root traits) of groundnut (Bhanumathi et al. 2005; Dogan et al. 2013; Lu et al. 2013; Bianucci et al. 2017; Zong et al. 2020). Cadmium toxicity (>200 μM) and lead (>1000 mg /L) has shown toxic effects on plant growth, chlorophyll content, lower antioxidant activity and higher MDA content (Dogan et al. 2013; Dong et al. 2020). Excess cadmium was found to reduce the root surface area, number of root tips and specific root length, however the root diameters increased considerably. The groundnut cultivars with fine roots showed a high capability of Cd accumulation (Lu et al. 2013). The negative effect of mercuric acetate on seedling growth, root and shoot length and chlorophyll content was observed in groundnut (Bhanumathi et al. 2005). Chromium accumulation is shown in the groundnut cultivars with an extensive root system (Zong et al. 2020). There is a need to study the effect of heavy metals and their accumulation in groundnut cultivars as they induce an adverse effect on human health upon consumption.

Several physiological, morphological and yield related traits are screened to know the impact of abiotic stresses on crops (Table 4.2).

Table 4.2 Important phenotypic traits screened for abiotic stress tolerance in groundnut

S. No.	Category	Traits or observations recorded
1	Leaf and leaf related traits	Specific leaf area; Specific Leaf weight; Leaf thickness; SPAD Chlorophyll Meter Reading; Leaf shape; Stomatal frequency/density; Chlorophyll fluorescence; Photosynthetic rate; Stomatal conductance; Transpiration rate; Intercellular CO_2 concentration; Relative water content; Specific leaf nitrogen content
2	Flower and flower related traits	Flower initiation; 50% flowering; Pollen viability; Stigma receptivity; Pollen growth
3	Shoot and shoot related traits	Plant height; Number of primary branches; Number of secondary branches; Canopy temperature
4	Root and root related traits	Root length; Root shoot ratio; Root structure
5	Yield related traits	Pod to flower ratio; Number of pods per plant; Leaf dry weight; Stem dry weight; Haulm weight; Pod weight; Seed weight; Seed weight; Test weight; Shell weight; Shelling percentage; Biological yield; Harvesting index; Sound mature kernel percentage
6	Seed and seed related traits	Germination percentage; Temperature induction response
7	Biochemical studies	Total chlorophyll content; Total carotenoid content; Total anthocyanin content; Membrane injury index; Chlorophyll stability index; Lipid peroxidation assay; Activity estimation of enzymatic antioxidants; ROS estimation

4.3 Genetic Resources for Exploring the Abiotic Stress Tolerance

Cultivated groundnut is believed to be monophyletic origin with low genetic diversity. *A. duranensis* (A-genome) and *A. ipanensis* (B-genome) are considered as the ancestors of cultivated groundnut. Wild relatives of the groundnut have gone through several rough environments and many have managed to survive till date. Even though, the yield and other commercial requirements are not fulfilled by wild spp., they exhibit tolerance/resistance to several abiotic and biotic stresses. This potential of the wild relatives can be tapped to improve cultivated groundnut. Hence, it is very much important to understand the classification of these genetic resources for its usage in breeding programs.

The genus *Arachis* has at least 81 described species (Stalker 2017) and are broadly classified under nine taxonomic sections (Krapovickas and Gregory 1994) based on their cytogenetic and morphological features along with their respective geographic distribution and sexual compatibilities. A gene pool can be defined as “The total genetic diversity found within a population or a species expected to have extensive diversity and better ablility to withstand the challenges posed by environmental stresses" (Valls and Simpson 2005).

Groundnut genetic resources are broadly classified as the primary (GP1), secondary (GP2) and tertiary (GP3) gene pools based on their sexual compatibility. GP1 includes species that can be directly mated with the crop to produce fertile progeny. The section *Arachis* contains the GP1 of cultivated groundnut with two tetraploids, *A. hypogaea* and *A. monticola* ($2n = 4x = 40$; genome AB). GP2 is composed of the most closely related wild species which on crossing with GP1 results in partial fertility. The GP2 of *A. hypogaea's* include its close relatives, diploid ancestor ($2n = 2x = 20$) with genome of A, B, F and K. GP3 is made up of even more distantly related crop wild species. They produce sterile hybrids on crossing with GP1 (Rami et al. 2014). The groundnut germplasm is conserved at national and international gene banks of ICRISAT, USDA-ARS and CAAS (Pandey et al. 2012b). Secondary and tertiary genepools of *Arachis* are known to have resistance/tolerance towards abiotic and biotic stresses (Simpson et al. 2003; Mallikarjuna et al. 2011; Fonceka et al. 2012; Stalker et al. 2013; Upadhyaya et al. 2014). There were several efforts to develop synthetic amphidiploids using the wild relatives of the cultivated groundnut (Burow et al. 2001; Mallikarjuna et al. 2011). Besides, synthetic amphidiploids and autotetraploids, chromosome segment substitution lines (CSSL) (Fonceka et al. 2012), targeting induced local lesions in genomes (TILLING) populations (Knoll et al. 2011), multi-parent populations like multiparent advanced generation intercross (MAGIC), nested association mapping (NAM) populations (Pandey et al. 2020b; Scott et al. 2020) are available today as important genetic resources of groundnut breeding. These genetic resources need to be explored to identify the genomic regions conferring tolerance to abiotic stresses in groundnut. The advanced genomics technologies could help harness the potentials of the available genetic resources in groundnut. For instance, TILLING by sequencing was used to identify the functions of new candidate genes in groundnut (Guo et al. 2015).

Over the years, studies have reported the availability of several genotypes identified as source of abiotic stress tolerance which is often governed by quite a lot of genes and environment (G × E) interactions. Drought being a major abiotic stress worldwide, resulting in loss of yield and quality of rainfed groundnut, several lines with superior performance under different kinds of drought (early, mid and end-season drought) have been identified and are now available for use in the breeding program after extensive field screening (Nigam et al. 2002; Monyo and Varshney 2016).

The studies on the groundnut improvement using transgenic methods are limited. Among the several stress-response genes, transcription factors (TFs) play a key role. Coexpression of stress-responsive TFs, *AtHB7*, *AtDREB2A* and *AtABF3* (associated with downstream gene expression) have showed improved tolerance to salinity, drought and oxidative stresses compared to wild types, with increased total biomass (Pruthvi et al. 2014). Transgenic groundnut plants with overexpression of gene *mtl*D (from *Escherichia coli*) involved in the biosynthesis of mannitol showed a better performance to several traits that govern abiotic stress tolerance (Patel et al. 2017).

4.3.1 Genomics in Abiotic Stress Tolerance Studies

The primary mandate of breeding programs is to develop cultivars with high yielding capacity, resistance to abiotic and biotic stresses and adaptation to specific environmental conditions. The groundnut breeders in the past have used mass-selection to make use of natural variation among local varieties. Negative correlation between disease resistance and yield makes mass selection non-preferable in groundnut (Knauft and Wynne 1995). The methodologies like mutagenesis and hybridization were utilized more frequently in crop improvement programs. The pedigree process, which enables the breeder to perform selection for highly heritable traits viz., pod size, shape, seed size, plant type and testa colour in early segregating populations, has become more widespread among groundnut breeders. This method greatly reduced the size of individual segregating populations. Later, SSD (single-seed descent) method and recurrent selection were utilized in groundnut (Wynne 1976; Hildebrand 1984). Backcross breeding methodologies have also been used in the past years as they aid in the detection of qualitatively inherited traits but are not commonly used due to the lack of purely inherited diseases and insect resistance traits. Genotype and environment interactions are widespread in groundnut hence, multi-year and multi-location testing is an essential part of a breeding program. With the availability of modern technology and tools, molecular intervention in breeding became evident. The genomics-assisted breeding strategies were proven to be more accurate and repeatable. In this regard, the crop improvement in groundnut has witnessed the utilization of modern tools in the past decade (Pandey et al. 2020a) including genomic selection for complex traits like crop yield under drought stress with high G × E condition (Pandey et al. 2020b). Crop improvement in the present era relies on a 5G breeding strategy that gives accuracy and increases productivity in breeding programmes. The 5Gs include utilising "genome, germplasm, gene function, genomic breeding, and genome editing in crop enhancement" (Varshney et al. 2020). The sequence information was utilized in developing the marker systems, diversity arrays and expression studies that can enable to identify the candidate genes that are conferring abiotic stress tolerance. The utilization of genomics resources along with the recent advances in artificial intelligence can hasten the process of crop improvement.

4.3.2 Diversity Analysis

Crop diversity is the variation observed in genetic and phenotypic characteristics of crop plants. It helps farmers and plant breeders to develop high yielding and more productive varieties. Crop diversification is one of the strategies in agricultural diversification which farmers have adopted to reduce risks and challenges involved in agriculture and to increase their farm income. Therefore, preservation of diverse crop germplasm serves as valuable source of variability for breeding. The greatest diversity of groundnuts is found in South America, which is also considered to be the centre of origin of the crop, where the Chaco region is known as the primary centre of diversity and other areas as secondary centres of diversity of cultivated groundnuts (Krapovickas 1969; Gregory and Gregory 1976).

Arachis hypogaea is divided into two subspecies, *hypogaea* and *fastigiata.* The subsp. *hypogaea* is characterized by alternate branching and absence of flowers on the main axis and has a long-life cycle, whereas subsp. *fastigiata* is acclaimed by sequential branching and the flowers over main axis with shorter life cycle. Further, subsp. *fastigiata* is subdivided into four botanical varieties, *vulgaris* (Spanish type), *fastigiata* (Valencia type)*, peruviana,* and *aequatoriana.* Subsp. *hypogaea* is subdivided into two botanical varieties, *hypogaea* (Virginia type) and *hirsuta* (Krapovickas 1969).

Morphological and agronomic characteristics are visible descriptors that serve as key factors for diversity studies. Identification of genotypes at the farm level can be easily done with the help of these morphological descriptors. Evaluation of phenotypic diversity for various morphological descriptors and agronomic traits like pod yield, number of pods per plant and late leaf spot resistance in groundnut core collection showed significant variation (Banla et al. 2020). The parameters such as width and length of seeds and pods and hundred seed weight were found to be higher in the *hypogaea* group than in the *fastigiata* group. The leaf length and width, shelling percentage, and plant height were higher in *fastigiata* group (Upadhyaya 2003).The number of pods per plant and yield per plant were highly variable while pod length and width were less variables in comparison with other traits (Banla et al. 2020).

The Asian core collection of groundnuts consisting of 504 accessions was evaluated for agronomic traits in two seasons at two locations (Swamy et al. 2003). Significant variation was seen in the characteristics related to flower initiation, number of primary and secondary branches, and other yield characteristics as mentioned in Table 4.2.

"Genetic diversity is essential to meet the diverse goals in plant breeding such as producing cultivars with increased yield, wider adaptation, desirable quality and pest resistance" (Nevo et al. 1982). Genetic divergence is also a pre-requisite for hybridization program or agronomic improvement of crop species to obtain desirable genotypes. "More diverse the parents, the greater are the chances for obtaining the highest heterotic F_1 and broad-spectrum variability in segregating generation" (Arunachalam 1981). Genus *Arachis* has a huge diversity of genomes. Out of 15 genomes (A, B, AB, D, F, K, EX, T, PR, H, C, T, E, R1 and R2) available in *Arachis* genus, reference genome sequences are available only for few genomes (A, B, and AB) (Stalker 2017).

Study on genetic variability and genetic divergence was carried out using 29 exotic and local lines of groundnut and were grouped into seven clusters based on characters like plant height, total pods per plant, kernels per pod, hundred pod weight, shelling percentage, HI and pod yield per plant (Islam et al. 2005). Reddy and Reddy (1993) also reported that hundred pod weight, number of branches per plant and HI accounted for more than 80% of total divergence.

In situ hybridization studies between wild species and *A. hypogaea* indicated the genome differentiation in section *Arachis* may be due to recurring elements (Seijo et al. 2007). Isozymes and proteins were used in the initial molecular diversity studies (Krishna and Mitra 1987; Grieshammer 1989; Lu and Pickersgill 1993), restriction fragment length polymorphism (RFLP; Kochert et al. 1991, 1996; Paik-Ro et al. 1992), random amplified polymorphic DNA (RAPD; Halward et al. 1991, 1992; Hilu and Stalker 1995; Subramanian et al. 2000; Dwivedi et al. 2001) and amplified fragment length polymorphism (AFLP; He and Prakash 1997, 2001; Gimenes et al. 2002; Herselman 2003; Milla et al. 2005). Higher levels of polymorphism were observed with microsatellites or simple sequence repeats (SSRs) in groundnut (Pandey et al. 2012a; Krishna et al. 2004; Moretzsohn et al. 2005; Barkley et al. 2007; Varshney et al. 2009).

The genome survey sequence and EST (expressed sequence tags) in *Arachis* were used to develop genomic and genic SSRs that are utilized to study molecular diversity (Ferguson et al. 2004; Cuc et al. 2008; Mondal et al. 2012; Bhad et al. 2016). Genomic SSR markers were used for molecular diversity, detection of association with diverse resistance (Mace et al. 2006; Mondal and Badigannavar 2010), and development of framework map in cultivated groundnut (Gautami et al. 2012c). The microsatellite markers were used to study diversity in several core and mini core collections available in groundnut (Wang et al. 2011; Jianget al. 2014; Mukri et al. 2012).

Besides, other marker systems like *Arachis hypogaea* transposable element (AhTE) markers (Bhat et al. 2008; Shirasawa et al. 2012; Gayathri et al. 2018), diversity arrays technology (DArT) markers have been developed in groundnut. Due to the availability of less expensive sequencing platforms, several research groups have generated and reported single nucleotide polymorphism (SNP) markers (Khera et al. 2013; Zhou et al. 2014; Chopra et al. 2015; Hong et al. 2015; Clevenger et al. 2017; Pandey et al. 2017; Peng et al. 2020).

Analysis of genetic diversity in the mini-core accessions of groundnut showed that oleic acid and protein content are two significant contributors towards genotype divergence. The oleic acid contributed 30.75% to the divergence of genotypes and protein content by 28.78% (Mukri et al. 2014). Genome wide association study (GWAS) for four physiological traits viz., leaf area index, canopy temperature, chlorophyll index, and normalized difference vegetative index in 125 accessions from ICRISAT groundnut mini core collection revealed that these traits are important in improving productivity and act as indirect indices offering drought tolerance (Shaibu et al. 2020). This study has also identified a total of 20 highly significant marker-trait associations (MTAs) with 11 SNP markers for four physiological traits of importance in groundnut.

In plants, microsatellites have been recognized as useful molecular markers in marker-assisted selection (MAS) and analysis of genetic divergence. In groundnut, RFLP, RAPD and AFLP markers have showed lower polymorphism between *A. hypogaea* and *A. monticola* (Kochert et al. 1996), but significant variations were reported among other *Arachis* species (Kochert et al. 1991; Pail-Ro et al. 1992) and between botanical varieties of cultivated groundnut using AFLP and DNA amplification fingerprinting (DAF) technique (He and Prakash 1997). Microsatellite markers are more preferred over other markers for construction of linkage maps and quantitative trait locus (QTL) analysis, as they are PCR-based, codominant, highly polymorphic and its ability to transfer among related species (Hougaard et al. 2008; Bertioli et al. 2009; Moretzsohn et al. 2009; Varshney et al. 2009).

Being allotetraploid in origin, the fundamental hardship for groundnut improvement is its narrow genetic base. During evolution, several diverse alleles that adapt to the ecological niches were acquired that can be utilized in crop improvement programmes. To ease the screening of underutilized wild germplasm sources, core collections of 831 and 1704 accessions of groundnut and a minicore of 112 and 184 accessions at USDA-ARS and ICRISAT, respectively (Holbrook et al. 1993; Upadhyaya et al. 2002). These core and mini core populations harbour greater diversity that can be efficiently utilized in breeding programs.

4.3.3 Molecular Mapping

With the advancements in genome sequencing, marker development and construction of genetic maps, QTL analysis has gathered a lot of valuable information that can be very well utilized for crop improvement programs in groundnut. Several marker systems like RFLP, RAPD, AFLP, DArT, SSR, AhTE and SNPs have been developed and were utilized for construction of maps, genetic diversity analysis, using markers for marker-assisted selection (MAS) and marker-assisted breeding (MAB) (reviewed in Pandey et al. 2012b; Janila et al. 2016; Bhat et al. 2020).

The mapping of associated genomic regions with target traits can be conducted by using multiple types of populations like recombinant inbred lines (RILs), near isogenic lines (NILs), F_2 population, backcross introgression lines, NAM, MAGIC and the natural populations in groundnut (Pandey et al. 2012b; Varshney et al. 2013; Janila et al. 2014; Pandey et al. 2020a). Based on the mapping population used, the trait mapping may be of three types viz., linkage mapping, linkage disequilibrium (LD) based GWAS, and joint linkage-association mapping (JLAM) (Pandey et al. 2016).

4.3.3.1 Linkage Mapping

Several attempts in constructing genetic linkage maps were made with diploid and tetraploid species of *Arachis* (Table 4.3). The first known genetic map with RFLP markers in *Arachis* species was constructed using F_2 population (*A. stenosperma* × *A. cardenasii*) resulting in eleven linkage groups (Halward et al. 1993). Later first generation of a synthetic interspecific tetraploid BC_1 population {[*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)]^{4x} × *A. hypogaea* (‘Florunner’)} was used to draft another RFLP-based linkage map (Burow et al. 2001). Other molecular markers such as AFLP (Herselman et al. 2004), RAPD (Garcia et al. 2005), SSR (Moretzsohn et al. 2009), DArT (Shasidhar et al. 2017) and SNP (Bertioli et al. 2014) were utilized to

Table 4.3 Availability of linkage maps in groundnut

S. No.	Parents	Mapping population used	Marker type	No. of markers used	Genome	Linkage groups obtained	Total map distance (cM)	Software used for linkage map construction	References
1	*A. stenosperma* × *A. cardenasii*	87 F_2	RFLP	132	AA	11	1063.00	Mapmaker	Halward et al. (1993)
2	*A. stenosperma* × *A. cardenasii*	11	RAPD, RFLP	800	AA	44	BC_1F_1	Mapmaker	Garcia et al. (2005)
3	*A. duranensis* × *A. stenosperma*	93 F_2	SSR	204	AA	11	1230.89	Mapmaker Macintosh version 2.0	Moretzsohn et al. (2005)
4	*A. duranensis* × *A. stenosperma*	93 F_2	SSR, Legume anchor marker, AFLPs, NBS homologs, SNPs, RGA-RFLPs, SCAR	369	AA	10	–	Mapmaker Macintosh version 2.0	Leal-Bertioli et al. (2009)
5	*A. duranensis* × *A. stenosperma*	89 F_5	SSR, TE	597	AA	10	544.00	JoinMap version 4	Shirasawa et al. (2013)
6	*A. duranensis* × *A. stenosperma*	90 F_5	SNP, SSR	384	AA	10	705.10	Mapmaker Macintosh version 2.0	Bertioli et al. (2014)
7	*A. duranensis* × *A. stenosperma*	93 F_6	SNP, SSR, RGA, anchor, morphological	502	AA	10	1004.10	JoinMap version 4; Mapmaker Macintosh version 2.0	Leal-Bertioli et al. (2016)

(continued)

Table 4.3 (continued)

S. No.	Parents	Mapping population used	Marker type	No. of markers used	Genome	Linkage groups obtained	Total map distance (cM)	Software used for linkage map construction	References
8	*A. duranensis* × *A. duranensis*	94 F_2	SNP, SSR, SSCP, RGC	1724	AA	10	1081.30	MSTMap; JoinMap 3.0	Nagy et al. (2012)
9	*A. ipaensis* × *A. magna*	93 F_2	SSR	149	BB	10	1294.00	Mapmaker Macintosh version 2.0	Moretzsohn et al. (2009)
10	*A. ipaensis* × *A. magna*	94 RILs	SSR, TE	798	BB	10	461.00	JoinMap; MapChart	Shirasawa et al. (2013)
11	*A. ipaensis* × *A. magna*	94 RILs	SSR, TE	399	BB	10	678.00	JoinMap®; Mapmaker; MapChart	Leal-Bertioli et al. (2015)
12	K 9484 × GKBSPSc 30081 In *A. batizocoi*	94 F_2	SSR	449	BB	16	1278.60	Mapmaker 3.0, Mapchart 2.2	Guo et al. (2012)
13	Florunner × TxAG-6 *[A. batizocoi*	78 BC_1F_1	RFLP	370	AABB	23	2210.00	Mapmaker/EXP v 3.0	Burow et al. (2001)
14	(*A. cardenasii* GKP10017 × *A. diogoi* GKP10602)]4 × }	78 BC_1F_1	SSR	91	AABB	22	1321.90	Rqtl package v.3.1.2 from R	Wilson et al. (2017)
15	ICG 12991 × ICGVSM 93541	60 F_2	AFLP	12	AABB	5	139.4	Mapmaker/EXP	Herselman et al. (2004)
16	[Fleur 11 × (*A. ipaensis* × *A. duranensis*)4 ×]	88 BC_1F_1	SSR	298	AABB	21	1843.70	Mapdisto software version 1.7.2.4	Foncéka et al. (2009)
17	Yueyou 13 × Zhenzhuhei	142 RILs	SSR	131	AABB	20	679.00	JoinMap 3.0 MapChart	Hong et al. (2008)

(continued)

Table 4.3 (continued)

S. No.	Parents	Mapping population used	Marker type	No. of markers used	Genome	Linkage groups obtained	Total map distance (cM)	Software used for linkage map construction	References
18	TAG 24 × ICGV 86031	318 RILs	SSR	135	AABB	22	1270.50	Mapmaker Macintosh version 2.0; MapChart	Varshney et al. (2009)
19	TAG 24 × ICGV 86031	318 RILs	SSR	191	AABB	22	1785.40	Mapmaker WinQTL Cartographer, version 2.5; Genotype Matrix Mapping (GMM) software ver. 2.1	Ravi et al. (2011)
20	Yueyou 13 × Zhenzhuhei	142 $F_{4:6}$	SSR	132	AABB	19	684.90	JoinMap 3.0 MapChart	Hong et al. (2010)
21	Yueyou 13 × Fu 95-5	84 $F_{4:6}$	SSR	109	AABB	21	540.69	JoinMap 3.0 MapChart	Hong et al. (2010)
22	Yueyou 13 × J11	136 $F_{4:6}$	SSR	46	AABB	13	401.70	JoinMap 3.0 MapChart	Hong et al. (2010)
23	TAG 24 × GPBD 4	268 RILs	SSR	56	AABB	14	462.24	Mapmaker/EXP; MapChart 2.2	Khedikar et al. (2010)
24	TAG 24 × GPBD 4	266 RILs	SSR	188	AABB	20	1922.40	Mapmaker/EXP v.3.0; MapChart 2.2	Sujay et al. (2012)
25	TAG 24 × GPBD 4	266 RILs	SSR, TE	289	AABB	20	1730.80	JoinMap 4	Kolekar et al. (2016)

(continued)

Table 4.3 (continued)

S. No.	Parents	Mapping population used	Marker type	No. of markers used	Genome	Linkage groups obtained	Total map distance (cM)	Software used for linkage map construction	References
26	TG 26 × GPBD 4	146 RILs	SSR	45	AABB	8	657.90	Mapmaker/EXP v.3.0; MapChart 2.2	Sarvamangala et al. (2011)
27	TG 26 × GPBD 4	146 RILs	SSR	181	AABB	21	1963.00	JoinMap 3.0 MapChart	Sujay et al. (2012)
28	ICGS 44 × ICGS 76	188 RILs	SSR	82	AABB	15	831.40	Mapmaker/EXP v.3.0	Gautami et al. (2012a)
29	ICGS 76 × CSMG 84-1	177 RILs	SSR	119	AABB	20	2208.20	Mapmaker/EXP v.3.0	Gautami et al. (2012b)
30	SunOleic 97R × NC94022	352 RILs	SSR, CAPS	172	AABB	22	920.70	JoinMap 3.0 MapChart	Qin et al. (2012)
31	SunOleic 97R × NC94022	352 RILs	SSR, CAPS	206	AABB	20	1780.60	JoinMap version 4; MapChart 2.2	Pandey et al. (2014)
32	SunOleic 97R × NC94022	352 RILs	SSR	248	AABB	21	1425.90	JoinMap version 4; MapChart 2.2	Khera et al. (2016)
33	Tifrunner × GT-C20	94 F_2	SSR	318	AABB	21	1674.40	JoinMap version 4	Wang et al. (2012)
34	Tifrunner × GT-C20	248 RILs	SSR, CAPS	239	AABB	26	1213.40	JoinMap version 4; MapChart 2.2	Qin et al. (2012)
35	Tifrunner × GT-C20	248 RILs	SSR, CAPS	378	AABB	20	2487.40	JoinMap version 4; MapChart 2.2	Pandey et al. (2014)
36	Tifrunner × GT-C20	248 RILs	SSR	418	AABB	20	1935.40	JoinMap version 4; MapChart 2.2	Pandey et al. (2017)

(continued)

Table 4.3 (continued)

S. No.	Parents	Mapping population used	Marker type	No. of markers used	Genome	Linkage groups obtained	Total map distance (cM)	Software used for linkage map construction	References
37	YI-0311 × Nakateyutaka	186 F_2	SSR, TE	326	AABB	19	1332.90	JoinMap version 4 MapChart 2.2; GeneMapper software (Applied Biosystems)	Shirasawa et al. (2012)
38	Satonoka × Kintoki	94 F_2	SSR, TE	1114	AABB	21	2166.40	JoinMap version 4; MapChart 2.2; GeneMapper software (Applied Biosystems)	Shirasawa et al. (2012
39	*A. hypogaea* "Runner IAC 886" × (A. *ipaensis* × A. *duranensis*)4x	91 RILs	SSR, TE	1469	AABB	20	1442.00	JoinMap version 4; MapChart 2.2; GeneMapper software (Applied Biosystems)	Shirasawa et al. (2012)
40	*A. hypogaea* "Runner IAC 886" × (A. *ipaensis* × A. *duranensis*)4x	89 F_6	SNP, SSR	772	AABB	20	1487.30	Mapmaker/EXP v.3.0	Bertioli et al. (2014)
41	Zhonghua 5 × ICGV 86699	166 RILs	SNP, SSR	1685	AABB	20	1446.70	JoinMap version 4; MapChart 2.0	Zhou et al. (2014)
42	VG 9514 × TAG 24	164 RILS	SSR	95	AABB	24	882.90	Mapmaker/EXP; MapChart 2.1	Mondal et al. (2012)
43	VG 9514 × TAG 24	164 RILS	SSR, ISSR, TE, RGC	190	AABB	21	1796.70	Mapmaker/EXP; MapChart 2.1	Mondal et al. (2014)

(continued)

Table 4.3 (continued)

S. No.	Parents	Mapping population used	Marker type	No. of markers used	Genome	Linkage groups obtained	Total map distance (cM)	Software used for linkage map construction	References
44	Zhonghua 10 × ICG12625	232 F_2	SSR	470	AABB	20	1877.30	JoinMap® version 3.0	Huang et al. (2015)
45	Zhonghua 10 × ICG12625	140 RILs	SSR, TE	1219	AABB	20	2038.75	JoinMap version 3.0	Huang et al. (2016)
46	Fuchuan Dahuasheng × ICG 6375	218 $F_{2:3}$	SSR	347	AABB	22	1675.60	JoinMap version 3.0	Chen, Jiao, et al. (2016)
47	Xuhua 13 × Zhonghua 6	282 $F_{2:3}$	SSR	228	AABB	22	1337.70	JoinMap version 3.0	Chen et al. (2016)
48	Florida- EP™ “113” × Georgia Valencia	163 F_2	SSR, SNP	30	AABB	1	157.80	JoinMap version 4.0	Tseng et al. (2016)
49	ICGV 00350 × ICGV 97045	268 F_2	DArT, DArTseq	1152	AABB	20	2423.12	JoinMap version 4; MapChart 2.0	Vishwakarma et al. (2016)
50	79266 × D893	151 RILs	SSR	231	AABB	23	905.18	JoinMap version 4.0	Li et al. (2017)
51	Yuanza 9102 × Xuzhou 68-4	195 RILs	SSR	830	AABB	20	1386.19	JoinMap version 4; MapChart 2.3	Luo et al. (2017b)
52	ICGV 07368 × ICGV 06420	184 F_2	DArT, SSR	854	AABB	20	3526.00	JoinMap version 4; MapChart	Shasidhar et al. (2017)
53	ICGV 06420 × SunOleic 95R	179 F_2	DArT, DArTseq	1435	AABB	20	1869.00	JoinMap version 4; MapChart	Shasidhar et al. (2017)
54	Tamrun OL07 × Tx964117	90 RILs	SNP	1211	AABB	20	–	MSTMAP software	Liang et al. (2017)

(continued)

Table 4.3 (continued)

S. No.	Parents	Mapping population used	Marker type	No. of markers used	Genome	Linkage groups obtained	Total map distance (cM)	Software used for linkage map construction	References
55	TMV 2 × TMV 2- NLM	432 RILS	AhTE	91	AABB	20	1205.66	JoinMap version 4; MapChart 2.2	Hake et al. (2017)
56	3 populations	–	SSR	175	Consensus	22	885.40	JoinMap 3.0 MapChart	Hong et al. (2010)
57	2 populations	–	SSR	225	Consensus	20	1152.90	JoinMap 3.0 MapChart	Sujay et al. (2012)
58	3 populations	–	SSR	293	Consensus	20	2840.80	Mapmaker/EXP v.3.0	Gautami et al. (2012c)
59	2 populations	–	SSR	324	Consensus	21	1352.10	JoinMap version 4; MapChart 2.2	Qin et al. (2012)
60	11 populations	–	SSR	897	Consensus	20	3863.60	Mapmaker/EXP; MapChart 2.2	Gautami et al. (2012c)
61	16 populations	–	SSR, TE	3693	Consensus	20	2651.00	JoinMap version 4; MapChart	Shirasawa et al. (2013)

Updated from Desmae et al. (2018)

construct the genetic maps on biparental mapping population. After a lot of initial research that has been conducted in the AA and BB genomes of the groundnut crop, scientists moved towards mapping the genome of the cultivated groundnut. Though the genome of the cultivated groundnut was not quite easy to understand with simple mapping since it is an allotetraploid crop. SSR based linkage maps have also been created from an interspecific F_2 population of the A-genome (*A. duranensis* and *A. stenosperma*) with 170 SSRs and 11 linkage groups (Moretzsohn et al. 2005). With the availability of larger number of markers (1469) studying the relationship between the wild genotypes and the cultivated peanut became common (Shirasawa et al. 2013). The ability of the SNP markers to obtain more productive information from the genome of the crop is evident. Mapping was much specific and detailed with the SNP markers. They were also being integrated into the genetic maps. Hong et al. (2010) developed six consensus maps with 175 loci. Several scientists later dedicatedly used the SNPs to construct the genetic maps viz., Shirasawa et al. (2013) mapped 3693 SNP loci across the groundnut genome which helped in the crop's characterization. Besides, a consensus map was developed using 16 segregating populations of various diverse genetic backgrounds enabled mapping with greater genome coverage (Shirasawa et al. 2013). Most of the works emphasized in mapping several loci in a single genetic map and utilize it for MAB (e.g., Hong et al. 2008, 2010; Foncéka et al. 2009; Wang et al. 2012; Shirasawa et al. 2013).

In a biparental population-based mapping, considering the amount of recombination events that occur during the development of the population, the localization of the quantitative trait loci (QTLs) is only between 10 and 20 cM intervals and detection of QTL mainly resides on the phenotypic variation in the population. These characters are considered as a limitation when a biparental population is considered for mapping.

Commonly used biparental populations like the F_2 population are easy for construction and estimation of both additive and dominant effects with the limitations of lesser power, limited recombination and the temporary nature of the population. When it comes to the utility of introgressing specific genes, the backcross (BC) population is considered the best, but it will be highly impossible to estimate the dominant effects. BC population also takes a lot of time unlike F_2 population-based mapping and the temporary nature of the population adds difficulty in the process. On the other hand, doubled haploid (DH) population is known for its immortality and easy replication along with rapid map construction abilities. Despite its advantage to bring rapid recombination, it's use is very limited due to expenses involved in technology. RIL population-based map construction has the best of recombination in the population which in turn provided abundant opportunities in mapping the genome generally with very less mortality percentage and easy in replication at the cost of much more time requirements and impossibility to estimate the dominant effects (Ren et al. 2017).

Several small effect (<10% phenotypic variation explained-PVE) and major effect QTLs (>10% PVE) were identified and reported for various types of traits viz., agronomic and yield component traits (Selvaraj et al. 2009; Hake et al. 2017; Luo et al. 2017a), biotic stress resistance (Khedikar et al. 2010; Kolekar et al. 2016; Zhou et al. 2016; Pandey et al. 2017), abiotic stress tolerance especially for drought-related traits (Varshney et al. 2009; Leal-Bertioli et al. 2016) and quality traits (Sarvamangala et al. 2011; Shasidhar et al. 2017).

The efforts to dissect the QTLs for drought tolerance related traits like leaf area, transpiration efficiency, chlorophyll meter reading (SCMR), carbon discrimination ratio and other yield-related traits in groundnut mapping population TAG 24 × ICGV 86031 identified some main effect QTLs and many epistatic QTLs, indicating the complexity of the drought tolerance related traits. The QTL mapping was carried out using several tools like QTL Cartographer, QTL Network and Genotype Matrix Mapping (Ravi et al. 2011). Further, a consensus map with three mapping populations viz., TAG 24 × ICGV 86031, ICGS 76 × CSMG 84-1 and ICGS 44 × ICGS 76 was utilized for identification of 153 main-QTLs and 25 epistatic QTLs with low to moderate phenotypic variance for drought tolerance related traits (Gautami et al. 2012c). This study suggested the utilization of marker-assisted recurrent selection (MARS) and genomic selection (GS) for crop improvement. However, for other abiotic stresses like high-temperature tolerance, efforts are currently being made to detect genomic regions using linkage mapping (JL 24 × 55–437) and bulk segregant transcriptome mapping approaches. The mapping studies on salinity tolerance are yet to be explored in groundnut. Recently, about 19 main effect QTLs were identified for drought tolerance and Fe chlorosis and identified several transcription factors like bHLH, MyB, NAM at the QTL region (Pandey et al. 2021). So far, not much emphasis has been paid on the mapping of salinity and cold tolerance in groundnut.

4.3.3.2 Association Mapping Studies

There are very limited studies to explore the MTAs based on GWAS for abiotic stress tolerance in groundnut. The reference collection of groundnut comprising of 300 accessions were genotyped with DArT and SSR markers and phenotyping for 50 important agronomic, disease, quality traits and drought tolerance related traits. The genotypes were phenotyped for several drought tolerance related traits in well-watered and water-stressed conditions and about 152 MTAs were detected in both conditions (Pandey et al. 2014b). However, the GWAS on other abiotic stresses like salinity, high temperature, heavy metals, etc. need to be explored further.

4.4 Marker-Assisted Breeding

The germplasm is the foundation to improve the genetic content of cultivars. In this direction, cultivated and wild genotypes of groundnut have been gathered, conserved and extensively characterized at institutes like ICRISAT, USDA-ARS and OCRI-CAAS. The core and mini-core collection were generated in groundnut and screened for traits like yield, quality, abiotic and abiotic stresses (Liao 2017). Marker-assisted breeding helps to transfer desired genes into a recipient genetic background and minimizes the growing cycles needed for recovery of recurrent parent genome. As the linkage and association-based studies indicated several small effect QTLs/MTAs associated with drought tolerance with low to moderate phenotypic variance explained, the marker-assisted back cross-breeding is not effective. Hence either MARS or GS approaches should be utilized to transfer the genomic regions for improving elite groundnut genotypes for drought tolerance. There are limited published reports on the introgression breeding for abiotic stress tolerance in groundnut. In one of the studies, introgression of alleles from the wild species *Arachis duranensis* and *A. batizocoi* was carried out to improve earliness, WUE, pod yield and photosynthetic traits in a set of lines derived from the cross of an induced allotetraploid and cultivated groundnut with selection under water stress (Dutra et al. 2018). Also, the abiotic stress-related traits in groundnut are complex with high environmental influence which has further been adversely impacted by climate change. For such traits, genomic prediction-based selection of plant progenies provides greater opportunity in attaining genetic gains in the field. A recent study on genomic selection in groundnut has performed comparative assessment for different GS models and pinpointed 'naïve interaction model' and 'naïve and informed interaction models' for high prediction accuracy for the complex traits with high genotype × environment interactions (Pandey et al. 2020b).

4.5 Multi-omics Approaches

Advancements in genomics and computational biology has given a bigger opportunity to combine the molecular and computational tools to better understand the functions of the genes. Several omics approaches like transcriptomics using complete transcriptome sequencing reveals the uniquely expressed genes concerning abiotic stresses. The sequencing-based approaches are also utilized in identifying molecular markers like SNPs and InDels. However, drafting a picture based only on the transcriptome of the crop can be biased, since even the translated proteins are still subjected to several post-translational modifications which might result in a different phenotype. With several new platforms having arrived viz., 2D gel (Ingel proteomics); liquid Chromatography with tandem mass spectrometry (LC–MS/MS) in solution proteomics, Electrospray Ionization (ESI)—LCMS & matrix-assisted laser desorption/ionization-time of fligh (MALDI-TOF) to generate reliable protein sequence data instead of previously used Edmund degradation sequencing studying the molecular mechanisms have become much better nowadays. These sequence data can be further analyzed deeply for several new pathway proteins and new components. This promises a great insight in future to identify the exact final product of a targeted gene within the plant system to understand the metabolic profiles temporally and spatially during stress (Handakumbura et al. 2017; Kumar et al. 2017).

The gene expression atlas has provided the information on network of genes expressed during different developmental stages of groundnut plant in *hypogaea* (Clevenger et al. 2016) and *fastigiata* (Sinha et al. 2020) subspecies of *Arachis*. A report on the interpretation of the transcriptome profile of two wild species, *A. duranensis* and *A. magna* identified eight candidate genes that shared identical expression profiles in response to drought conditions and recovery at multiple stages. The genes such as *NAC* and *bZIP1* were annotated to be involved in signalling in response to drought in *A. duranensis* roots. Other genes that are involved in primary metabolism (*CA* or *NIT*), cell protection/ adaptation mechanisms (*CDSP, DiP,* or *EXLB*) were also reported in both *A. duranensis* and *A. magna* (Brasileiro et al. 2015).

An attempt to isolate drought-responsive genes from groundnut roots using suppression subtractive hybridization showed that 80 of the 111 transcripts had homology with known genes and 31 with unknown/ uncharacterized genes (Ding et al. 2014). This study also showed the involvement of *ANN, ADH* and *MnSOD* in the drought tolerance mechanism in groundnut. Also, the expression of *P5CS,* a key regulatory enzyme in proline biosynthesis, was increased in the tissues under drought stress (Ding et al. 2014). Genes encoding *lea3, lea4* and *metallothionein-like protein* had shown involvement in drought stress in peanuts (Su et al. 2011; Quan et al. 2007). The transcription factors like AP2/EREBP (AhWSI 279), bHLH (AhWSI 40), bZIP (AhWSI 20), CCAAT box (AhWSI 117), Homeobox (AhWSI6 11), Jumonji (AhWSI 72, AhWSI 116), NAC (AhWSI 153, AhWSI 308) and several zinc finger protein transcripts were induced under drought treatments in groundnut. Among the stress-related proteins, LEAs, HSPs, transcription factors like zinc fingers, AP2, Myb, WRKY and NAC and drought-induced protein were highly expressed in plants exposed to moderate levels of stress (Govind et al. 2009). Transcription factors (TFs) that play key role in eliciting stress responses in *A. duranensis* include MYB (13%), bZIP (13%), AP2-EREB and bHLH (8%), WRK (6%) and NAC (7%). In the case of *A. stenosperma* distribution of TFs were slightly different, that is MYB (14%), bZIP (18%), WRK (4%) bHLH (6%) and AP2-EREB (10%). The most expressed TF family in drought-imposed *A. duranensis* and fungi infected *A. stenosperma* leaves was basic leucine zipper (bZIP)-type TF protein which is followed by the MYB family (Guimaraes et al. 2012).

Oflate, a genome-wide transcriptome analysis was carried out on Valencia genotypes of groundnut C76-16 and Val-C, and the study indicated activation of key genes in ABA and sucrose metabolism pathways under drought stress conditions (Bhogireddy et al. 2020). Transcriptome analysis of salinity-affected groundnut genotypes has provided insights of the expression of transcription factors, genes related to cell wall biogenesis, cell growth, antioxidant activity etc., upon salinity stress (Zhang et al. 2020).

4.6 Genomics Aided Breeding

Conventional breeding methodologies have been followed since ages for the improvement of cultivars. For instance, ICGV 91114 and Dh 256 cultivars were released for drought-prone areas in India using conventional breeding approaches. However, the advancement in omics technologies has overcome these drawbacks of the conventional breeding approaches by easy understanding of the molecular mechanisms governing complex traits. These omics technologies coupled with next-generation sequencing technologies have resulted in higher genetic gains with high precision and accuracy in less time and resources (Fig. 4.2).

The wild germplasm sources harbor regions of the genome that show resistance to various environment stresses along with increased nutritional contents. Several institutes maintain genetic resources of groundnut namely, the NBPGR, DGR and the Gene Banks of ICRISAT in India; USDA, Texas A&M University, and North Carolina State University in the USA; OCRI CAAS and CRIGAAS; INTA and IBONE in Argentina and EMBRAPA -CENARGEN and the Instituto Agronomico de Campinas in Brazil.

The peanut genome sequencing was initiated by the International Peanut Genome Initiative (IPGI) in the year 2010 through the Peanut Genome Consortium (PGC). This consortium has sequenced two diploid progenitors, *A. duranensis* V14167 and *A. ipaensis* K30076 (Bertioli et al. 2016). In the same duration another initiative, Diploid Progenitor Peanut A-Genome Sequencing Consortium (DPPAGSC) has sequenced different A-genome genotype, PI475845 (Chen et al. 2016). While the genome size of A-genome was predicted to be 1.05 Gb and 1.21 Gb by DPPAGSC and IPGI, respectively. The genome size of B-genome was predicted to be 1.51 Gb with 41,840

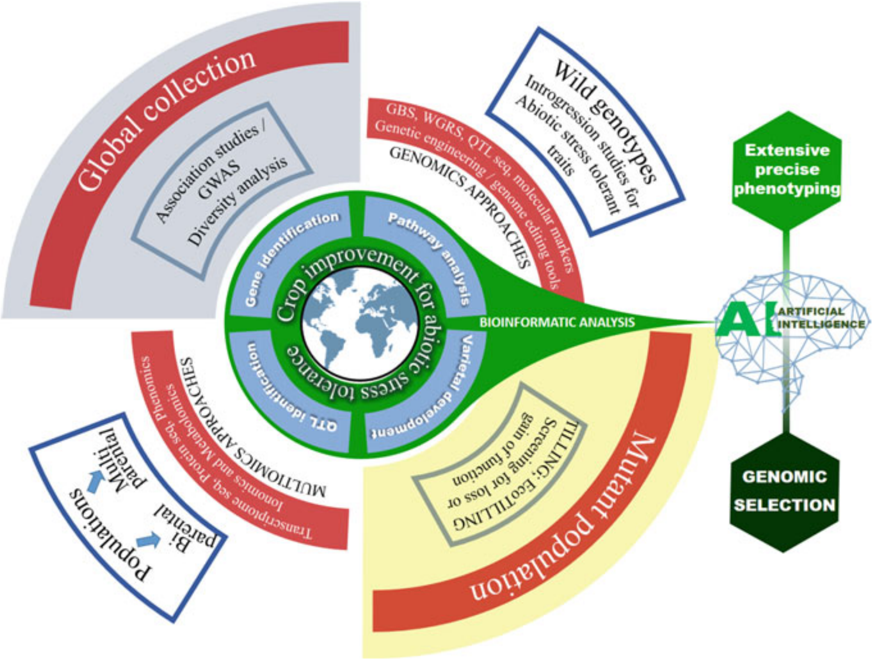

Fig. 4.2 Genomics approaches for abiotic stress tolerance in groundnut

genes by IPGI. This information on genome sequences will accelerate the research on gene discovery, trait mapping using genomics and molecular breeding tools.

Several cultivars with enhanced yield and resistance traits have been successfully developed using conventional breeding approaches but it is limited in improving few selective traits. Such traits can be specifically targeted and improved using molecular breeding approaches. Genomics has the potential to accelerate the processes of gene discovery, trait dissection and molecular breeding. Hence genomics-assisted breeding plays key role in the improvement of cultivars much faster than conventional breeding. For genetic analysis and trait association studies, accurate phenotyping data for target traits is equally important along with the marker data. The efficiency and accuracy of candidate gene discovery and trait mapping are positively correlated with the precise trait characterization in diverse genotypes (Varshney et al. 2018).

Genomics, a breakthrough technology serves as a promising tool for decrypting the stress responsiveness of crop plants with adaptation traits or in identifying underlying alleles or genes or QTLs in wild relatives. The stress adaptation of crop plants can be enhanced by molecular breeding approaches. Next-generation sequencing technologies and phenotyping platforms have metamorphosed molecular breeding to genomics-assisted breeding. Evaluation of genetic diversity and development of genetic linkage map are key steps in marker-assisted breeding programs (Dwivedi et al. 2003). Also, genomics-assisted breeding is found to have a key role in the development of climate change resilient crops (Kole et al. 2015; Gangrude et al. 2019).

Marker-assisted backcrossing (MABC) is a preferred molecular breeding approach for improving elite genotypes that are deficient in one or two important traits. Since majority of the economically important traits related to abiotic stresses are quantitative and governed by several small effect QTLs, an alternative molecular breeding method called MARS was suggested to boost such dynamic traits like yield and resistance to abiotic stress (Ribaut and Ragot 2007). Today, in groundnuts, MABC is used for traits linked to biotic stress tolerance (viz*.,* foliar diseases, root-knot nematodes) and to improve the oil content for which high phenotypic QTL markers have been successfully identified (Simpson et al. 2003; Chu et al. 2011). Both MABC and MARS require the design of family-based mapping populations and the discovery of QTLs/markers that are related to their respective traits. In case of unavailability of family-based mapping population and with polygenic traits 'genomic selection' (GS) could be an appropriate molecular breeding approach for crop improvement (Nayak et al. 2017; Chaudhari et al. 2019; Pandey et al. 2020). The data analysis for linkage and QTL mapping can be carried out by using software like Mapmaker, AntMap, DGMAP, JoinMap, MadMapper, Map Manager QTX, MSTMAP, RECORD, THREaD Mapper, R/qtl, R/qtl2 packages are utilized for linkage mapping and QTL analysis.

4.7 Genetic Engineering Approaches

Transgenic approaches seem to be an alternative solution in addressing the unsolvable issues regarding any stress for a plant. When the traditional breeding methods cannot be used to introduce genes, which impart tolerance to the concerned problem, the transgenic approach can be utilized. This approach allows us to explore and exploit genes across genepools irrespective of the genus. Several transgenic works have been made in groundnut in view of obtaining better yield, better seed quality, good oil quality and composition and various biotic and abiotic stress tolerance traits. Abiotic stress tolerance traits viz., mercury stress, salinity stress, drought, heat, etc., have been evaluated in the field level (Mallikarjuna et al. 2016; Gantait and Mondal 2018). The list of studies related to the use of transgenic approaches to impact tolerance to abiotic stresses is presented in Table 4.4.

4.8 Role of Bioinformatics as a Tool in Groundnut Genomics

The scientific advances in the areas of microsensors and microfluidics have witnessed the development of the next-generation and third-generation sequencing that enabled high-throughput data generation. Bioinformatics and computational biology have become an integral part of genomics and proteomics.

In groundnut genomics studies have reached new heights with the availability of genome sequences of diploid ancestors (Bertioli et al. 2016; Chen et al. 2016). The genome information have enabled large-scale genome-wide discovery of 515,223 Insertion/deletions (InDels, Vishwakarma et al. 2017) and millions of SSRs (Chen et al. 2016; Zhao et al. 2017; Luo et al. 2017b). A high-quality genome assembly of 'Tifrunner', a cultivated tetraploid genome has been sequenced (Bertioli et al. 2019). Another reference genome assembly was developed for *A. hypogaea* var. Shitouqi and whole-genome resequencing of 52 accessions of groundnut has provided foresight about the legume genomics, karyotypes, polyploidy evolution and the crop domestication of cultivated groundnut (Zhuang et al. 2019).

The genome sequences, markers and other genomics information has been deposited in a common crop specific database called as PeanutBase. The key goal of the PeanutBase is to combine genetic and genomic data to allow for quicker crop improvement in groundnut. Its aim is to compile, archive and make available genetic, genomic and gene expression resources for groundnut and to assist in breeding and molecular study.

The genetic resources on several groundnut species viz., *A. batizocoi, A. cardenasii, A. correntina, A. diogoi, A. duranensis, A. ipaensis, A. magna, A. hypogaea, A. monticola* and *A. stenosperma* is available in the database. It also allows to perform different BLAST options in the genome of the available three species viz., *A. duranensis, A. ipaensis* and *A. hypogaea.* The genome visualization platforms like

Table 4.4 Transgenic studies carried out for abiotic stress tolerance in groundnut

Groundnut genotype	Transgene & promoter	Gene origin	Methodology	Improvised characteristics or traits	References
Georgia runner	*merA* *ACT2*	*Arabidopsis thaliana*	Microprojectile bombardment	Mercury stress	Yang et al. (2003)
JL 24	*AtDREB1A* *35S CaMV*	*A. thaliana*	*Agrobacterium*-mediated	Drought stress	Bhatnagar-Mathur et al. (2007)
RD2, RD11, and RD3 from RD2, RD11 and RD33	*AtDREB1A* *35S CaMV*	*A. thaliana*	*Agrobacterium*-mediated	Drought stress	Vadez et al. (2007)
Georgia Green	*Bcl-xL* *CaMV 35*	*Homo sapiens*	*Microprojectile bombardment*	Paraquat and salinity tolerance	Chu et al. (2008)
Golden BARI-2000	*AtNHX1* *CaMV 35*	*A. thaliana*	*Agrobacterium*-mediated	Salinity and drought stress	Asif et al. (2011)
New Mexico Valencia A	*IPT* *CaMV 35*	*Agrobacterium tumefaciens*	*Agrobacterium*-mediated	Drought stress	Qin et al. (2011)
Golden and BARI-2000	*AtNHX1* 35S CaMV	*A. thaliana*	*Agrobacterium*-mediated	Salinity and drought stress	Banjara et al. (2012)
JL 24	*AtDREB1A* *rd29*	*A. thaliana*	*Agrobacterium*-mediated	Drought stress	Vadez et al. (2013)
JL 24	*AtDREB1A* *Rd29A*	*A. thaliana*	*Agrobacterium*-mediated	Drought stress	Bhatnagar-Mathur et al. (2014)
GG-20	*mtlD* CaMV35S	*Escherichia coli*	*Agrobacterium*-mediated	Drought stress	Patel et al. (2017)
K-134	PDH*45* *CaMV 35*	*Pisum sativum*	*Agrobacterium*-mediated (*in planta*)	Drought stress	Manjulatha et al. (2014)

(continued)

Table 4.4 (continued)

Groundnut genotype	Transgene & promoter	Gene origin	Methodology	Improvised characteristics or traits	References
Narayani	*MuNAC4* CaMV35S	*Macrotyloma uniflorum*	*Agrobacterium*-mediated	Drought stress	Pandurangaiah et al. (2014)
K-134	*AtNAC2* (*ANAC092*) CaMV35S	*A. thaliana*	*Agrobacterium*-mediated (*in planta*)	Salinity and drought stress	Patil et al. (2014)
TMV 2	*AtDREB2A*, *AtHB7* and *AtABF3* CaMV35S	*A. thaliana*	*Agrobacterium*-mediated	Salinity and drought stress	Pruthvi et al. (2014)
GG 20	*AtDREB1A*	*A. thaliana*	*Agrobacterium*-mediated	Salinity and drought stress	Sarkar et al. (2014)
GG-20	*SbpPAX* CaMV35S	*Salicornia brachiata*	*Agrobacterium*-mediated	Salinity stress	Singh et al. (2014)
GG-20	*SbASR-1* CaMV35S	*S. brachiata*	*Agrobacterium*-mediated	Salinity and drought stress	Tiwari et al. (2015)
GPBD 4	*Alfin1*, *PgHSF4*, and *PDH45* CaMV35S	Alfalfa, *Pennisetum glaucum*, Pea	*Agrobacterium*-mediated (*in planta*)	Drought stress	Ramu et al. (2016)
TMV 2	*AtDREB1A* CaMV35S	*A. thaliana*	*Agrobacterium*-mediated	Drought stress	Sarkar et al. (2016)
JL 24	*AtHDG11* Atrd29A	*A. thaliana*	*Agrobacterium*-mediated	Salinity and drought stress	Banavath et al. (2018)

GBrowser and JBrowser are integrated in the PeanutBase along with synteny and navigation tools.

The gene expression atlas, the data has been generated by comparing the RNA sequencing (RNA-Seq) read counts from 22 *A. hypogaea* tissues against the *A. ipaensis* and *A. duranensis* genome combined. It provides information on differentially expressed genes from each tissue comparison. Other information such as the nematode expression experiment and the drought expression experiment was also documented in this database (Dash et al. 2016). The published genotypic and phenotypic data are made available to the researchers working on groundnut. An interactive pedigree chart and a platform to compare the genotypes based on the heredity have also been made available in the database.

Resources for the peanut research community include:

1. PeanutBase: http://peanutbase.org/
2. American Peanut Council (coordinating the Peanut Genomics Initiative) http://www.peanutsusa.com/
3. American Peanut Research and Education Society (APRES), http://apresinc.com/
4. Peanut Information Network System (PINS), https://site.caes.uga.edu/pins/) https://peanuts.caes.uga.edu/
5. IBP—peanut; see trait dictionary https://www.integratedbreeding.net/cropinformation/groundnuts
6. Crop Ontology—peanut http://www.cropontology.org/ontology/CO_337/Groundnut
7. CGIAR Generation Challenge Program—peanut, http://www.generationcp.org/gcp-research/research-initiatives/legumes/legumes-groundnuts
8. PeanutDB, http://bioinfolab.muohio.edu/txid3818v1/

4.9 Conclusions

With effect of climate change, the abiotic stresses pose a major challenge in plant growth and sustainability of several crop species including groundnut. The groundnut crop must adapt to several abiotic stresses mainly to high temperature, drought and salinity. The abiotic stresses not only affect the crop yield by direct physiological effects but also impart a favourable environment for new pests and diseases. Hence it is very important to breed for the groundnut varieties tolerant to abiotic stresses with high productivity to address the food security and malnutrition prevailing in the developing countries. With the advancement in genomics research and computational know-hows, the genomics enabled crop improvement is possible in groundnut. Several efforts have been successful in imparting disease resistance in groundnut using genomics assisted approaches like MABC, MAS, QTL sequencing, etc. The studies on abiotic stress tolerance are limited to drought and efforts are being made for other stresses. With the current climate change scenario, it is necessary to study the mechanisms involved in abiotic stress tolerance, their cross-talks and improve the elite cultivars for better-withstanding capacity. Advanced genomics approaches like genomic selection and genotype prediction based on artificial intelligence can be utilized to improve the groundnut crop for complex traits related to abiotic stress tolerance.

Acknowledgements The authors are thankful to Department of Biotechnology (DBT), Indian Council of Agricultural Research (ICAR)- National Agricultural Science Funds (NASF) and AdaptNET (Strengthening education, research and innovation for climate smart crops in India), an ERASMUS+-CAPACITY BUILDING project, funded by the European Commission for financial support and trainings.

References

Abd El-Rheem Kh M, Safi-Naz S (2015) Effect of soil salinity on growth, yield and nutrient balance of peanut plants. Intl J ChemTech Res8 (12):564–568

Akter S, Jharna DY, Mali SK, Sayeed A (2020) Salinity induced deleterious effects on germination, growth, physiological and biochemical process of two varieties of groundnut (*Arachis hypogaea* L.). Arch Agric Environ Sci 5 (2):144–150

Arunachalam V (1981) Genetic distance in plant breeding. Indian J Genet 41(2):226–236

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141(2):391–396

Asif MA, Zafar Y, Iqbal J, Iqbal MM, Rashid U, Ali GM, Arif A, Nazir F (2011) Enhanced expression of AtNHX1, in transgenic groundnut (*Arachis hypogaea* L.) improves salt and drought tolerance. Mol Biotechnol 49 (3):250–256

Awal MA, Ikeda T (2002) Effects of changes in soil temperature on seedling emergence and phenological development in field-grown stands of peanut (*Arachis hypogaea*). Environ Exp Bot 47:101–113

Azad M, Kalam A, Hamid M, Rafii MY, Malek M (2014) Combining ability of pod yield and related traits of groundnut (*Arachis hypogaea* L.) under salinity stress. Sci World J 2014

Balota M, Isleib TG, Tallury S (2012) Variability for drought related traits of Virginia-type peanut cultivars and advanced breeding lines. Crop Sci 52(6):2702–2713

Banavath JN, Chakradhar T, Pandit V, Konduru S, Guduru KK, Akila CS, Podha S, Puli CO (2018) Stress inducible overexpression of AtHDG11 leads to improved drought and salt stress tolerance in peanut (*Arachis hypogaea* L.). Front Chem 6:34

Banjara M, Zhu L, Shen G, Payton P, Zhang H (2012) Expression of an Arabidopsis sodium/proton antiporter gene (AtNHX1) in peanut to improve salt tolerance. Plant Biotechnol Rep 6(1):59–67

Banla EM, Dzidzienyo DK, Diangar MM, Melomey LD, Offei SK, Tongoona P, Desmae H (2020) Molecular and phenotypic diversity of groundnut (*Arachis hypogaea* L.) cultivars in Togo. Physiol Mol Biol Plants 26 (7):1489–1504

Barkley NA, Dean RE, Pittman RN, Wang ML, Holbrook CC, Pederson GA (2007) Genetic diversity of cultivated and wild-type peanuts evaluated with M13-tailed SSR markers and sequencing. Genet Res 89(2):93–106

Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EK, Liu X, Gao D, Clevenger J, Dash S et al (2016) The genome sequences of *Arachis duranensis* and *Arachis ipaensis*, the diploid ancestors of cultivated peanut. Nat Genet 48(4):438–446

Bertioli DJ, Ozias-Akins P, Chu Y, Dantas KM, Santos SP, Gouvea E, Guimarães PM, Leal-Bertioli SC, Knapp SJ, Moretzsohn MC (2014) The use of SNP markers for linkage mapping in diploid and tetraploid peanuts. G3: Genes Genomes Genet 4(1):89–96

Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli SC, Ren L, Farmer AD, Pandey MK et al (2019) The genome sequence of segmental allotetraploid peanut *Arachis hypogaea.* Nat Genet 51(5):877–884

Bhad PG, Mondal S, Badigannavar AM (2016) Genetic diversity in groundnut (*Arachis hypogaea* L.) genotypes and detection of marker trait associations for plant habit and seed size using genomic and genic SSRs. J Crop Sci Biotechnol 19 (3):203–221

Bhagat N, Dayal D, Acharya D (1992) Performance of Spanish peanuts during winter-summer season at two locations in India. Trop Agri 69:93–95

Bhanumathi P, Ganesan M, Jayabalan N (2005) Physiological effect of organic mercury on the growth of peanut (*Arachis hypogaea* L.) seedlings. Plant Arch 5:665–669

Bhat R, Patil V, Chandrashekar T, Sujay V, Gowda M, Kuruvinashetti M (2008) Recovering flanking sequence tags of a miniature inverted-repeat transposable element by thermal asymmetric interlaced-PCR in peanut. Curr Sci 95(4):452–453

Bhat RS, Shirasawa K, Monden Y, Yamashita H, Tahara M (2020) Developing transposable element marker system for molecular breeding. In: Jain M, Garg R (eds) Legume genomics: methods and protocols. Humana, New York, pp 233–251

Bhatnagar-Mathur P, Devi MJ, Reddy DS, Lavanya M, Vadez V, Serraj R, Yamaguchi-Shinozaki K, Sharma KK (2007) Stress-inducible expression of *At DREB1A* in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency under water-limiting conditions. Plant Cell Rep 26 (12):2071–2082

Bhatnagar-Mathur P, Rao JS, Vadez V, Dumbala SR, Rathore A, Yamaguchi-Shinozaki K, Sharma KK (2014) Transgenic peanut overexpressing the DREB1A transcription factor has higher yields under drought stress. Mol Breed 33(2):327–340

Bhogireddy S, Xavier A, Garg V, Layland N, Arias R, Payton P, Nayak SN, Pandey MK, Puppala N, Varshney RK (2020) Genome-wide transcriptome and physiological analyses provide new insights into peanut drought response mechanisms. Sci Rep 10(1):1–16

Bianucci E, Furlan A, del Carmen TM, Hernández LE, Carpena-Ruiz RO, Castro S (2017) Antioxidant responses of peanut roots exposed to realistic groundwater doses of arsenate: Identification of glutathione S-transferase as a suitable biomarker for metalloid toxicity. Chemosphere 181:551–561

Boontang S, Girdthai T, Jogloy S, Akkasaeng C, Vorasoot N, Patanothai A, Tantisuwichwong N (2010) Responses of released cultivars of peanut to terminal drought for traits related to drought tolerance. Asian J Plant Sci 9(7):423–431

Brasileiro AC, Morgante CV, Araujo AC, Leal-Bertioli SC, Silva AK, Martins AC, Vinson CC, Santos CM, Bonfim O, Togawa RC (2015) Transcriptome profiling of wild *Arachis* from water-limited environments uncovers drought tolerance candidate *genes.* Plant Mol Biol Rep 33(6):1876–1892

Burow MD, Simpson CE, Starr JL, Paterson AH (2001) Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (*Arachis hypogaea* L.): broadening the gene pool of a monophyletic polyploid species. Genetics 159 (2):823–837

Carvalho M, Vorasoot N, Puppala N, Muitia A, Jogloy S (2017) Effects of terminal drought on growth, yield and yield components in Valencia peanut genotypes. SABRAO J Breed Genet 49(3):270–279

Chaudhari S, Khare D, Patel SC, Subramaniam S, Variath MT, Sudini HK, Singh SM, Bhat RS, Janila P (2019) Genotype × environment studies on resistance to late leaf spot and rust in genomic selection training population of peanut (*Arachis hypogaea* L.). Front Plant Sci 10:1338

Chauhan YS, Senboku T (1997) Evaluation of groundnut genotypes for heat tolerance 1. Ann Appl Biol 131(3):481–489

Chen W, Jiao Y, Cheng L, Huang L, Liao B, Tang M, Ren X, Zhou X, Chen Y, Jiang H (2016) Quantitative trait locus analysis for pod-and kernel-related traits in the cultivated peanut (*Arachis hypogaea* L.). BMC Genetics 17(1):25

Chopra R, Burow G, Farmer A, Mudge J, Simpson CE, Wilkins TA, Baring MR, Puppala N, Chamberlin KD, Burow MD (2015) Next-generation transcriptome sequencing, SNP discovery and validation in four market classes of peanut *Arachis hypogaea L.*. Mol Genet Genom 290(3):1169–1180

Chu Y, Wu C, Holbrook C, Tillman B, Person G, Ozias-Akins P (2011) Marker-assisted selection to pyramid nematode resistance and the high oleic trait in peanut. Plant Genome 4(2):110–117

Chu Y, Deng X, Faustinelli P, Ozias-Akins P (2008) Bcl-xL transformed peanut (*Arachis hypogaea* L.) exhibits paraquat tolerance. Plant Cell Rep 27 (1):85–92

Clevenger J, Chu Y, Scheffler B, Ozias-Akins P (2016) A developmental transcriptome map for allotetraploid *Arachis hypogaea.* Front Plant Sci 7:1446

Clevenger J Chu Y, Chavarro C, Agarwal G, Bertioli DJ, Leal-Bertioli SC, Pandey MK, Vaughn J, AbernathyB Barkley NA, Hovav R (2017) Genome-wide SNP genotyping resolves signatures ofselection and tetrasomic recombination in peanut. Mol plant 10(2):309–322

Craufurd PQ, Prasad PVV, Kakani VG, Wheeler TR, Nigam SN (2003) Heat tolerance in groundnut. Field Crops Res 80(1):63–77

Craufurd PQ, Prasad PVV, Waliyar F, Taheri A (2006) Drought, Pod yield, Pre-harvest Aspergillus infection and Aflatoxin contamination on peanut in Niger. Field Crops Res 98:20–29

Cuc LM, Mace ES, Crouch JH, Quang VD, Long TD, Varshney RK (2008) Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). BMC Plant Biol 8(1):55

Dash S, Cannon EK, Kalberer SR, Farmer AD, Cannon SB (2016) PeanutBase and other bioinformatic resources for peanut. In: Peanuts. Elsevier, pp 241–252

Desmae H, Janila P, Okori P, Pandey MK, Motagi BN, Monyo E, Mponda O, Okello D, Sako D, Echeckwu C (2018) Genetics, genomics and breeding of groundnut (*Arachis hypogaea* L.). Plant Breed 138(4):425–444

Devi MJ, Sinclair TR, Vadez V (2010) Genotypic variability among peanut (*Arachis hypogea* L.) in sensitivity of nitrogen fixation to soil drying. Plant Soil 330 (1–2):139–148

Dilip S, Singh S (2000) Response of summer groundnut (*Arachis hypogaea*) and succeeding maize (Zea mays) to sulphur and phosphorus fertilization. Indian J Agric Sci 70(10):657–660

Ding H, Zhang ZM, Qin FF, Dai LX, Li CJ, Ci DW, Song WW (2014) Isolation and characterization of drought-responsive genes from peanut roots by suppression subtractive hybridization. Elec J Biotechnol 17(6):304–310

Dogan M, Akgul H, Tozlu I (2013) Lead accumulation and toxicity in peanut (*Arachis hypogaea* L.) seedlings. Fresenius Environ Bull 22 (8):2350–2356

Dong Y, Chen W, Liu F, Wan Y (2020) Physiological responses of peanut seedlings to exposure to low or high cadmium concentration and the alleviating effect of exogenous nitric oxide to high cadmium concentration stress. Plant Biosyst 154(3):405–412

Dutra WF, Guerra YL, Ramos JP, Fernandes PD, Silva CR, Bertioli DJ, Leal-Bertioli SC, Santos RC (2018) Introgression of wild alleles into the tetraploid peanut crop to improve water use efficiency, earliness and yield. PLoS One 13 (6):e0198776

Dwivedi S, Gurtu S, Chandra S, Yuejin W, Nigam S (2001) Assessment of genetic diversity among selected groundnut germplasm. I: RAPD analysis. Plant Breed 120(4):345–349

Dwivedi S, Crouch J, Nigam S, Ferguson M, Paterson A (2003) Molecular breeding of groundnut for enhanced productivity and food security in the semi-arid tropics: opportunities and challenges. Adv Agro 80:153–221

Ekeleme F, Kwari J, Omoigui L, Chikoye D (2011) Phosphorus effects on growth and yield of groundnut varieties in the tropical savannas of northeast Nigeria. J Trop Agri 49:25–30

Evans RG, Sadler EJ (2008) Methods and technologies to improve efficiency of water use. Water Resour Res 44(7)

FAOSTAT (2019) Food and Agriculture Organization of the United Nations Statistical Databases, Rome, Italy. http://www.fao.org/faostat/en/#data/QC. Accessed on 20 Jan 2021

Ferguson ME, Burow MD, Schulze S, Bramel P, Paterson A, Kresovich S, Mitchell S (2004) Microsatellite identification and characterization in peanut (*A. hypogaea* L.). Theor Appl Genet 108(6):1064–1070

Foncéka D, Hodo-Abalo T, Rivallan R, Faye I, Sall MN, Ndoye O, Fávero AP, Bertioli DJ, Glaszmann J-C, Courtois B (2009) Genetic mapping of wild introgressions into cultivated peanut: a way toward enlarging the genetic basis of a recent allotetraploid. BMC Plant Biol 9(1):1–13

Fonceka D, Tossim H-A, Rivallan R, Vignes H, Lacut E, De Bellis F, Faye I, Ndoye O, Leal-Bertioli SC, Valls JF (2012) Construction of chromosome segment substitution lines in peanut (*Arachis hypogaea* L.) using a wild synthetic and QTL mapping for plant morphology. PLoS One 7(11): e48642

Fountain J, Scully B, Ni X, Kemerait R, Lee D, Chen Z-Y, Guo B (2014) Environmental influences on maize-*Aspergillus flavus* interactions and aflatoxin production. Front Microbiol 5:40

Gangurde SS, Kumar R, Pandey AK, Burow M, Laza HE, Nayak SN, Guo B, Liao B, Bhat RS, Madhuri N (2019) Climate-smart groundnuts for achieving high productivity and improved quality: current status, challenges, and opportunities. In: Kole C (ed) Genomic designing of climate-smart oilseed crops. Springer, Cham, pp 133–172

Gantait S, Mondal S (2018) Transgenic approaches for genetic improvement in groundnut (*Arachis hypogaea* L.) against major biotic and abiotic stress *factors.* J Genet Eng Biotechnol 16(2):537–544

Garcia G, Stalker H, Schroeder E, Lyerly J, Kochert G (2005) A RAPD-based linkage map of peanut based on a backcross population between the two diploid species *Arachis stenosperma* and *A. cardenasii.* Peanut Sci 32(1):1–8

Gautami B, Fonceka D, Pandey MK, Moretzsohn MC, Sujay V, Qin H, Hong Y, Faye I, Chen X, BhanuPrakash A (2012a) An international reference consensus genetic map with 897 marker loci based on 11 mapping populations for tetraploid groundnut (*Arachis hypogaea* L.). PloS One 7(7): e41213

Gautami B, Pandey M, Vadez V, Nigam S, Ratnakumar P, Krishnamurthy L, Radhakrishnan T, Gowda M, Narasu M, Hoisington D (2012b) QTL analysis and consensus genetic map for drought tolerance traits based on three RIL populations of cultivated groundnut (*Arachis hypogaea* L.). Mol Breed 32:757–772

Gautami B, Pandey MK, Vadez V, Nigam SN, Ratnakumar P, Krishnamurthy L, Radhakrishnan T, Gowda MVC, Narasu ML, Hoisington DA, Knapp SJ, Varshney RK (2012c) Quantitative trait locus analysis and construction of consensus genetic map for drought tolerance traits based on three recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). Mol Breed 30:757–772

Gayathri M, Shirasawa K, Varshney R, Pandey M, Bhat R (2018) Development of AhMITE1 markers through genome-wide analysis in peanut (*Arachis hypogaea* L.). BMC Res Notes 11(1):10

Gimenes MA, Lopes CR, Valls JF (2002) Genetic relationships among Arachis species based on AFLP. Genet Mol Biol 25(3):349–353

Giorno F, Wolters-Arts M, Mariani C, Rieu I (2013) Ensuring reproduction at high temperatures: the heat stress response during anther and pollen development. Plants 2(3):489–506

Govind G, ThammeGowda HV, Kalaiarasi PJ, Iyer DR, Muthappa SK, Nese S, Makarla UK (2009) Identification and functional validation of a unique set of drought induced genes preferentially expressed in response to gradual water stress in peanut. Mol Genet Genom 281(6):591–605

Gregory WC, Gregory MP (1976) Groundnut. In: Simmonds NW (ed) Evolution of crop plants. Longman, London, pp 151–154

Gregory W, Krapovickas A, Gregory MP (1980) Structure, variation, evolution, and classification in Arachis. Structure, variation, evolution, and classification in Arachis. In: Summerfield RJ, Bunting AH (eds) Advances in legume sciences. Kew Royal Botanic Gardens, vol 2, pp 469–481

Grieshammer U (1989) Isozymes in peanuts: variability among US cultivars and Mendelian and non-Mendelian inheritance. MS thesis, North Carolina State University, Raleigh, NC

Guimarães PM, Brasileiro AC, Morgante CV, Martins AC, Pappas G, Silva OB, Togawa R, Leal-Bertioli SC, Araujo AC, Moretzsohn MC (2012) Global transcriptome analysis of two wild relatives of peanut under drought and fungi infection. BMC Genomics 13(1):387

Guo B, Chen ZY, Lee RD, Scully BT (2008) Drought stress and preharvest aflatoxin contamination in agricultural commodity: genetics, genomics and proteomics. J Integr Plant Biol 50(10):1281–1291

Guo Y, Khanal S, Tang S, Bowers JE, Heesacker AF, Khalilian N, Nagy ED, Zhang D, Taylor CA, Stalker HT (2012) Comparative mapping in intraspecific populations uncovers a high degree of macrosynteny between A-and B-genome diploid species of peanut. BMC Genomics 13(1):1–12

Guo Y, Abernathy B, Zeng Y, Ozias-Akins P (2015) TILLING by sequencing to identify induced mutations in stress resistance genes of peanut (*Arachis hypogaea*). BMC Genomics 16(1):157

Hake AA, Shirasawa K, Yadawad A, Sukruth M, Patil M, Nayak SN, Lingaraju S, Patil P, Nadaf H, Gowda M (2017) Mapping of important taxonomic and productivity traits using genic and non-genic transposable element markers in peanut (*Arachis hypogaea* L.). PLoS One 12(10): e0186113

Halward TM, Stalker HT, Larue EA, Kochert G (1991) Genetic variation detectable with molecular markers among unadapted germ-plasm resources of cultivated peanut and related wild species. Genome 34(6):1013–1020

Halward T, Stalker H, Kochert G (1993) Development of an RFLP linkage map in diploid peanut species. Theor Appl Genet 87(3):379–384

Halward T, Stalker T, LaRue E, Kochert G (1992) Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol Biol 18 (2):315–325

Hamidou F, Ratnakumar P, Halilou O, Mponda O, Kapewa T, Monyo E, Faye I, Ntare B, Nigam S, Upadhyaya H (2012) Selection of intermittent drought tolerant lines across years and locations in the reference collection of groundnut (*Arachis hypogaea* L.). Field Crops Res 126:189–199

Hamidou F, Halilou O, Vadez V (2013) Assessment of groundnut under combined heat and drought stress. J Agro Crop Sci 199(1):1–11

Hamidou F, Rathore A, Waliyar F, Vadez V (2014) Although drought intensity increases aflatoxin contamination, drought tolerance does not lead to less aflatoxin contamination. Field Crops Res 156:103–110

Handakumbura PP, Hixson KK, Purvine SO, Jansson C, Paša-Tolić L (2017) Plant iTRAQ-Based Prote*omics*. Curr Prot Plant Biol 2(2):158–172

Hatfield JL, Prueger JH (2015) Temperature extremes: effect on plant growth and development. Wea Clim Extremes 10:4–10

He G, Prakash CS (1997) Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). Euphytica 97(2):143–149

He G, Prakash C (2001) Evaluation of genetic relationships among botanical varieties of cultivated peanut (*Arachis hypogaea* L.) using AFLP markers. Genet Resour Crop Evol 48 (4):347–352

Herselman L, Thwaites R, Kimmins F, Courtois B, Van Der Merwe P, Seal SE (2004) Identification and mapping of AFLP markers linked to peanut (*Arachis hypogaea* L.) resistance to the aphid vector of groundnut rosette disease. Theor Appl Genet 109(7):1426–1433

Herselman L (2003) Genetic variation among Southern African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. Euphytica 133(3):319–327

Hildebrand G (1984) Use of the single-seed descent method of selection in groundnut breeding in Zimbabwe. In: Regional Groundnut Workshop for Southern Africa, 26:137

Hilu K, Stalker H (1995) Genetic relationships between peanut and wild species of *Arachis* sect. Arachis (Fabaceae): Evidence from RAPDs. Plant Syst Evol 198(3–4):167–178

Holbrook CC, Stalker HT (2010) Peanut breeding and genetic resources. In: Janick J (ed) Plant breeding reviews, vol 22. Wiley, pp 297–356

Holbrook CC, Anderson WF, Pittman RN (1993) Selection of a core collection from the US germplasm collection of peanut. Crop Sci 33(4):859–861

Holbrook C, Isleib T, Ozias-Akins P, Chu Y, Knapp S, Tillman B, Guo B, Gill R, Burow M (2013) Development and phenotyping of recombinant inbred line (RIL) populations for peanut (*Arachis hypogaea*). Peanut Sci 40(2):89–94

Hong Y-B, Liang X-Q, Chen X-P, Liu H-Y, Zhou G-Y, Li S-X, Wen S-J (2008) Construction of genetic linkage map based on SSR markers in peanut (*Arachis hypogaea* L.). Agric Sci China 7(8):915–921

Hong Y, Chen X, Liang X, Liu H, Zhou G, Li S, Wen S, Holbrook CC, Guo B (2010) A SSR-based composite genetic linkage map for the cultivated peanut (*Arachis hypogaea* L.) genome. BMC Plant Biol 10(1):17

Hong Y, Pandey MK, Liu Y, Chen X, Liu H, Varshney RK, Liang X, Huang S. (2015) Identification and evaluation of single-nucleotide polymorphisms in allotetraploidpeanut (*Arachishypogaea* L.) based on amplicon sequencing combined with high resolution melting (HRM) analysis. Front Plant Sci 2:1068

Hougaard BK, Madsen LH, Sandal N, de Carvalho MM, Fredslund J, Schauser L, Nielsen AM, Rohde T, Sato S, Tabata S (2008) Legume anchor markers link syntenic regions between *Phaseolus vulgaris*, *Lotus japonicus, Medicago truncatula* and *Arachis.* Genetics 179(4):2299–2312

Huang B, Rachmilevitch S, Xu J (2012) Root carbon and protein metabolism associated with heat tolerance. J Exp Bot 63(9):3455–3465

Huang L, He H, Chen W, Ren X, Chen Y, Zhou X, Xia Y, Wang X, Jiang X, Liao B (2015) Quantitative trait locus analysis of agronomic and quality-related traits in cultivated peanut (*Arachis hypogaea* L.). Theor Appl Genet 128(6):1103–1115

Huang L, Ren X, Wu B, Li X, Chen W, Zhou X, Chen Y, Pandey MK, Jiao Y, Luo H (2016) Development and deployment of a high-density linkage map identified quantitative trait loci for plant height in peanut (*Arachis hypogaea* L.). Sci Rep 6:39478

Hundal S, Kaur P (1996) Climate change and its impact on crop productivity in Punjab, India. Climate Variability and Agriculture. Narosa Publishing, New Delhi, India, pp 377–393

Islam M, Alam S, Islam M, Hossain M (2005) Genetic variability and diversity of groundnut (*Arachis hypogaea* L.). Agriculturist 3:96–103

Jain VK (2000) Fundamental of plant physiology. S. Chand Publishing, ISBN: 8121904625

Janila P, Nigam S, Abhishek R, Anil Kumar V, Manohar S, Venuprasad R (2014) Iron and zinc concentrations in peanut (*Arachis hypogaea* L.) seeds and their relationship with other nutritional and yield parameters. J Agric Sci 153(6):975–994

Janila P, Pandey MK, Shasidhar Y, Variath MT, Sriswathi M, Khera P, Manohar SS, Nagesh P, Vishwakarma MK, Mishra GP (2016a) Molecular breeding for introgression of fatty acid desaturase mutant alleles (ahFAD2A and ahFAD2B) enhances oil quality in high and low oil containing peanut genotypes. Plant Sci 242:203–213

Janila P, Variath MT, Pandey MK, Desmae H, Motagi BN, Okori P, Manohar SS, Rathnakumar A, Radhakrishnan T, Liao B (2016b) Genomic tools in groundnut breeding program: status and perspectives. Front Plant Sci 7:289

Jiang H, Huang L, Ren X, Chen Y, Zhou X, Xia Y, Huang J, Lei Y, Yan L, Wan L (2014) Diversity characterization and association analysis of agronomic traits in a Chinese peanut (*Arachis hypogaea* L.) mini-core collection. J Integr Plant Biol 56(2):159–169

Jongrungklang N, Jogloya S, Kesmala T, Vorasoot N, Patanothai A (2014) Responses of rooting traits in peanut genotypes under pre-flowering drought stress. Intl J Plant Prod 8:335–352

Kamara AY, Ekeleme F, Kwari JD, Omoigui LO, Chikoye D (2011) Phosphorus effects on growth and yield of groundnut varieties in the tropical savannas of northeast Nigeria. J Trop Agri 49:25–30

Kambiranda DM, Vasanthaiah HK, Katam R, Ananga A, Basha SM, Naik K (2011) Impact of drought stress on peanut (*Arachis hypogaea* L.) productivity and food safety. Plants Environ 249–272

Kashiwagi J, Krishnamurthy L, Crouch J, Serraj R (2006) Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Field Crops Res 95(2–3):171–181

Khedikar Y, Gowda M, Sarvamangala C, Patgar K, Upadhyaya H, Varshney R (2010) A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theor Appl Genet 121(5):971–984

Khera P, Pandey MK, Wang H, Feng S, Qiao L, Culbreath AK, Kale S, Wang J, Holbrook CC, Zhuang W (2016) Mapping quantitative trait loci of resistance to tomato spotted wilt virus and leaf spots in a recombinant inbred line population of peanut (*Arachis hypogaea* L.) from SunOleic 97R and NC94022. PLoS One 11(7):e0158452

Khera P, Upadhyaya HD, Pandey MK, Roorkiwal M, Sriswathi M, Janila P, Guo Y, McKain MR, Nagy ED, Knapp SJ, Leebens-Mack J (2013) Single nucleotide polymorphism–based genetic diversity in the reference set of peanut (*Arachis spp.*) by developing and applying cost-effective kompetitive allele specific polymerase chain reaction genotyping assays. Plant Genome 6(3):1–11

Klimešová J, Holková L, Středa T (2020) Drought stress response in maize: molecular, morphological and physiological analysis of tolerant and sensitive genotypes. Maydica 65(1):9

Knauft DA, Wynne JC (1995) Peanut breeding and genetics. In: Advances in agronomy, vol 55. Elsevier, pp 393–445

Knoll JE, Ramos ML, Zeng Y, Holbrook CC, Chow M, Chen S, Maleki S, Bhattacharya A, Ozias-Akins P (2011) TILLING for allergen reduction and improvement of quality traits in peanut (*Arachis hypogaea* L.). BMC Plant Biol 11(1):81

Kochert G, Stalker HT, Gimenes M, Galgaro L, Lopes CR, Moore K (1996) RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). Amer J Bot 83(10):1282–1291

Kochert G, Halward T, Branch W, Simpson C (1991) RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor Appl Genet 81(5):565–570

Kole C, Muthamilarasan M, Henry R, Edwards D, Sharma R, Abberton M, Batley J, Bentley A, Blakeney M, Bryant J (2015) Application of genomics-assisted breeding for generation of climate resilient crops: progress and prospects. Front Plant Sci 6:563

Kolekar RM, Sujay V, Shirasawa K, Sukruth M, Khedikar YP, Gowda MVC, Pandey MK, Varshney RK, Bhat RS (2016) QTL mapping for late leaf spot and rust resistance using an improved genetic map and extensive phenotypic data on a recombinant inbred line population in peanut (*Arachis hypogaea* L.). Euphytica 209(1):147–156

Koolachart R, Jogloy S, Vorasoot N, Wongkaew S, Holbrook C, Jongrungklang N, Kesmala T, Patanothai A (2013) Rooting traits of peanut genotypes with different yield responses to terminal drought. Field Crops Res 149:366–378

Krapovickas A, Gregory WC (1994) Taxonomia del genero Arachis (Leguminosae). Bonplandia VIII:1–187

Krapovickas A (1969) The origin. variability and spread of the groundnut (Arachis hypogaea). In: The domestication and exploitation of plants and animals. Gerald Duckworth & Co, London, pp 427–441

Krishna GK, Zhang J, Burow M, Pittman RN, Delikostadinov SG, Lu Y, Puppala N (2004) Genetic diversity analysis in Valencia peanut (*Arachis hypogaea* L.) using microsatellite markers. Cell Mol Biol Lett 9:685–697

Krishna T, Mitra R (1987) Arachin polymorphism in groundnut (*Arachis hypogaea* L.). Phytochemistry 26(4):897–902

Kumar R, Bohra A, Pandey AK, Pandey MK, Kumar A (2017) Metabolomics for plant improvement: Status and prospects. Front Plant Sci 8:1302

Lamaoui M, Jemo M, Datla R, Bekkaoui F (2018) Heat and drought stresses in crops and approaches for their mitigation. Front Chem 6:26

Leal-Bertioli SC, José ACV, Alves-Freitas DM, Moretzsohn MC, Guimarães PM, Nielen S, Vidigal BS, Pereira RW, Pike J, Fávero AP (2009) Identification of candidate genome regions controlling disease resistance in *Arachis*. BMC Plant Biol 9(1):1–12

Leal-Bertioli SC, Cavalcante U, Gouvea EG, Ballén-Taborda C, Shirasawa K, Guimarães PM, Jackson SA, Bertioli DJ, Moretzsohn MC (2015) Identification of QTLs for rust resistance in the peanut wild species Arachis magna and the development of KASP markers for marker-assisted selection. Genes Genomes Genet 5(7):1403–1413

Leal-Bertioli SC, Moretzsohn MC, Roberts PA, Ballén-Taborda C, Borba TC, Valdisser PA, Vianello RP, Araújo ACG, Guimarães PM, Bertioli DJ (2016) Genetic mapping of resistance to

Meloidogyne arenaria in Arachis stenosperma: a new source of nematode resistance for peanut. Genes Genomes Genet 6(2):377–390

Leal-Bertioli SCM, Bertioli DJ, Guimarães PM, Pereira TD, Galhardo I, da Silva J, Brasileiro ACM, Oliveira RS, SILVA P, Vadez V (2012) The effect of tetraploidization of wild Arachis on leaf morphology and other drought-related traits. Embrapa Recursos Genéticos e Biotecnologia-Artigo em periódico indexado (ALICE)

Levitt J (1972) Responses of plants to environmental stresses. Academic Press, New York, p 697

Levitt J (1980) Responses of plants to environmental stress: chilling, freezing, and high temperature stresses. Academic Press, New York

Li Y, Li L, Zhang X, Zhang K, Ma D, Liu J, Wang X, Liu F, Wan Y (2017) QTL mapping and marker analysis of main stem height and the first lateral branch length in peanut (*Arachis hypogaea* L.). Euphytica 213(2):57

Liang Y, Baring M, Wang S, Septiningsih EM (2017) Mapping QTLs for leafspot resistance in peanut using SNP-based next-generation sequencing markers. Plant Breed Biotechnol 5(2):115–122

Liao B (2017) Germplasm characterization and trait discovery in peanut. In: Varshney RK, Pandey MK, Puppala N (eds) The peanut genome. Springer, ham, pp 53–68

Liao B, Zhou R, Lei Y, Li D (2000) Evaluation of tolerance to aluminum toxicity in high-yielding groundnut genotypes. Chin J OilCrop Sci 22(1):38–42, 45

Lu Z, Zhang Z, Su Y, Liu C, Shi G (2013) Cultivar variation in morphological response of peanut roots to cadmium stress and its relation to cadmium accumulation. Ecotoxicol Environ Saf 91:147–155

Lu J, Pickersgill B (1993) Isozyme variation and species relationships in peanut and its wild relatives (*Arachis* L.—Leguminosae). Theor Appl Genet 85 (5):550–560

Luo H, Ren X, Li Z, Xu Z, Li X, Huang L, Zhou X, Chen Y, Chen W, Lei Y (2017a) Co-localization of major quantitative trait loci for pod size and weight to a 3.7 cM interval on chromosome A05 in cultivated peanut (*Arachis hypogaea* L.). BMC Genomics 18(1):58

Luo H, Xu Z, Li Z, Li X, Lv J, Ren X, Huang L, Zhou X, Chen Y, Yu J (2017b) Development of SSR markers and identification of major quantitative trait loci controlling shelling percentage in cultivated peanut (*Arachis hypogaea* L.). Theor Appl Genet 130(8):1635–1648

Mace E, Phong D, Upadhyaya H, Chandra S, Crouch J (2006) SSR analysis of cultivated groundnut (*Arachis hypogaea* L.) germplasm resistant to rust and late leaf spot diseases. Euphytica 152(3):317–330

Mallikarjuna G, Rao TSRB, Kirti P (2016) Genetic engineering for peanut improvement: current status and prospects. Plant Cell Tiss Org Cult (PCTOC) 125(3):399–416

Mallikarjuna N, Senthilvel S, Hoisington D (2011) Development of new sources of tetraploid Arachis to broaden the genetic base of cultivated groundnut (*Arachis hypogaea* L.). Genet Resour Crop Evol 58(6):889–907

Manjulatha M, Sreevathsa R, Kumar AM, Sudhakar C, Prasad T, Tuteja N, Udayakumar M (2014) Overexpression of a pea DNA helicase (PDH45) in peanut (*Arachis hypogaea* L.) confers improvement of cellular level tolerance and productivity under drought stress. Mol Biotechnol 56(2):111–125

Matsui T, Singh B (2003) Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp Agri 39(1):29

Meena H, Bhaduri D, Yadav R, Jain N, Meena M (2017) Agronomic performance and nutrient accumulation behaviour in groundnut-cluster bean cropping system as influenced by irrigation water salinity. Proc Natl Acad Sci India Sect B Biol Sci 87(1):31–37

Mensah J, Akomeah P, Ikhajiagbe B, Ekpekurede E (2006) Effects of salinity on germination, growth and yield of five groundnut genotypes. Afr J Biotechnol 5(20):1973–1979. https://www.ajol.info/index.php/ajb/article/view/55923

Milla S, Isleib T, Stalker H (2005) Taxonomic relationships among Arachis sect. Arachis species as revealed by AFLP markers. Genome 48(1):1–11

Mondal S, Badigannavar A (2010) Molecular diversity and association of SSR markers to rust and late leaf spot resistance in cultivated groundnut (*Arachis hypogaea* L.). Plant Breed 129(1):68–71

Mondal S, Badigannavar A, D'Souza S (2012) Development of genic molecular markers linked to a rust resistance gene in cultivated groundnut (*Arachis hypogaea* L.). Euphytica 188(2):163–173

Mondal S, Hadapad AB, Hande PA, Badigannavar AM (2014) Identification of quantitative trait loci for bruchid (Caryedon serratus Olivier) resistance components in cultivated groundnut (*Arachis hypogaea* L.). Mol Breed 33(4):961–973

Monyo ES, Varshney R (2016) Seven seasons of learning and engaging smallholder farmers in the drought-prone areas of sub-Saharan Africa and South Asia through Tropical Legumes, 2007–2014. ICRISAT, Pattancheru, Hyderabad, India

Moretzsohn M, Leoi L, Proite K, Guimaraes P, Leal-Bertioli S, Gimenes M, Martins W, Valls J, Grattapaglia D, Bertioli D (2005) A microsatellite-based, gene-rich linkage map for the AA genome of *Arachis* (Fabaceae). Theor Appl Genet 111(6):1060–1071

Moretzsohn MC, Barbosa AV, Alves-Freitas DM, Teixeira C, Leal-Bertioli SC, Guimarães PM, Pereira RW, Lopes CR, Cavallari MM, Valls JF (2009) A linkage map for the B-genome of Arachis (Fabaceae) and its synteny to the A-genome. BMC Plant Biol 9(1):1–10

Mukri G, Nadaf HL, Bhat RS, Gowda M, Upadhyaya HD, Sujay V (2012) Phenotypic and molecular dissection of ICRISAT mini core collection of peanut (*Arachis hypogaea* L.) for high oleic acid. Plant Breed 131(3):418–422

Mukri G, Nadaf H, Gowda M, Bhat R, Upadhyaya H (2014) Genetic diversity analysis based on nutritional, oil quality and yield component traits in mini core collection of groundnut (*Arachis hypogaea* L.). Karnataka J Agric Sci 27(02):219–221

Nagy ED, Guo Y, Tang S, Bowers JE, Okashah RA, Taylor CA, Zhang D, Khanal S, Heesacker AF, Khalilian N (2012) A high-density genetic map of Arachis duranensis, a diploid ancestor of cultivated peanut. BMC Genomics 13(1):469

Nautiyal P, Rajgopal K, Zala P, Pujari DS, Basu M, Dhadhal BA, Nandre BM (2008) Evaluation of wild Arachis species for abiotic stress tolerance: I. Thermal stress and leaf water relations. Euphytica 159(1–2):43–57

Nayak SN, Pandey MK, Jackson SA, Liang X, Varshney RK (2017) Sequencing ancestor diploid genomes for enhanced genome understanding and peanut improvement. In: Varshney RK, Pandey MK, Puppala N (eds) The peanut genome. Springer, Cham, pp 135–147

Nevo E, Golenberg E, Beiles A, Brown A, Zohary D (1982) Genetic diversity and environmental associations of wild wheat, Triticum dicoccoides, in Israel. Theor Appl Genet 62(3):241–254

Nigam S, Chandra S, Sridevi KR, Bhukta M, Reddy A, Rachaputi NR, Wright G, Reddy P, Deshmukh M, Mathur R (2005) Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Ann Appl Biol 146(4):433–439

Nigam S, Nageswara Rao R, Wright G (2002) Field Screening for drought tolerance in groundnut. In: Field screening for drought tolerance proceedings of an international workshop on international crops research institute for the semi-arid tropics, Patancheru, Andhra Pradesh, India, pp 147–151. ISBN 92-9066-448-7

Ntare B, Williams J, Dougbedji F (2001) Evaluation of groundnut genotypes for heat tolerance under field conditions in a Sahelian environment using a simple physiological model for yield. J Agric Sci 136(1):81–88

Paik-Ro O, Smith R, Knauft D (1992) Restriction fragment length polymorphism evaluation of six peanut species within the Avachis section. Theor Appl Genet 84(1–2):201–208

Pandey MK, Gautami B, Jayakumar T, Sriswathi M, Upadhyaya HD, Gowda MVC, Radhakrishnan T, Bertioli DJ, Knapp SJ, Cook DR (2012a) Highly informative genic and genomic SSR markers to facilitate molecular breeding in cultivated groundnut (*Arachis hypogaea*). Plant Breed 131(1):139–147

Pandey MK, Monyo E, Ozias-Akins P, Liang X, Guimarães P, Nigam SN, Upadhyaya HD, Janila P, Zhang X, Guo B (2012b) Advances in Arachis genomics for peanut improvement. Biotechnol Adv 30(3):639–651

Pandey MK, Roorkiwal M, Singh V, Lingam A, Kudapa H, Thudi M, Chitikineni A, Rathore A, Varshney RK (2016) Emerging genomic tools for legume breeding: current status and future perspectives. Front Plant Sci 7:455

Pandey MK, Pandey AK, Kumar R, Nwosu CV, Guo B, Wright GC, Bhat RS, Chen X, Bera SK, Yuan M, Jiang H, Faye I, Radhakrishnan T, Wang X, Liang X, Liao B, Zhang X, Varshney RK, Zhuang W (2020a) Translational genomics for achieving higher genetic gains in groundnut. Theor Appl Genet 133:1679–1702

Pandey MK, Chaudhari S, Jarquin D, Janila P, Crossa J, Patil SC, Sundravadana S, Khare D, Bhat RS, Radhakrishnan T, Hickey JM, Varshney RK (2020b) Genome-based trait prediction in multi-environment breeding trials in groundnut. Theor Appl Genet 133:3101–3117

Pandey MK, Gangurde SS, Sharma V, Pattanashetti SK, Naidu GK, Faye I, Hamidou F, Desmae H, Kane NA, Yuan M, Vadez V, Nigam SN, Varshney RK (2021) Improved genetic map identified major QTLs for drought tolerance and iron deficiency tolerance related traits in groundnut. Genes 12:37

Pandey MK, Upadhyaya HD, Rathore A, Vadez V, Sheshshayee MS, Sriswathi M, Govil M, Kumar A, Gowda MVC, Sharma S, Hamidou F, Anil Kumar V, Khera P, Bhat RS, Khan AW, Singh S, Li H, Monyo E, Nadaf HL, Mukri G, Jackson SA, Guo B, Liang X, Varshney RK (2014b) Genome-wide association studies for 50 agronomic traits in groundnut using the reference set comprising 300 genotypes from 48 countries of the semi-arid tropics of the world. PLoS One 9(8):e105228

Pandey MK, Wang ML, Qiao L, Feng S, Khera P, Wang H, Tonnis B, Barkley N, Wang J, Holbrook C, Culbreadth A, Varshney RK, Guo B (2014a) Identification of QTLs associated with peanut oil content and mapping FAD2 genes and their relative contribution to oil quality in peanut (*Arachis hypogaea* L.). BMC Genetics 15:133

Pandey MK, Wang ML, Qiao L, Feng S, Khera P, Wang H, Tonnis B, Barkley NA, Wang J, Holbrook CC (2014b) Identification of QTLs associated with oil content and mapping FAD2 genes and their relative contribution to oil quality in peanut (*Arachis hypogaea* L.). BMC Genetics 15(1):133

Pandey MK, Wang H, Khera P, Vishwakarma MK, Kale SM, Culbreath AK, Holbrook CC, Wang X, Varshney RK, Guo B (2017) Genetic dissection of novel QTLs for resistance to leaf spots and tomato spotted wilt virus in peanut (*Arachis hypogaea* L.). Front Plant Sci 8:25

Pandurangaiah M, Rao GL, Sudhakarbabu O, Nareshkumar A, Kiranmai K, Lokesh U, Thapa G, Sudhakar C (2014) Overexpression of horsegram (*Macrotylomauniflorum* Lam. Verdc.) NAC transcriptional factor (MuNAC4) in groundnut confers enhanced drought tolerance. Mol Biotechnol 56(8):758–769

Patel KG, Thankappan R, Mishra GP, Mandaliya VB, Kumar A, Dobaria JR. (2017) Transgenic peanut (*Arachis hypogaea* L.) overexpressing mtlD gene showed improved photosynthetic, physio-biochemical, and yield-parameters under soil-moisture deficit Stress in lysimeter system. Front Plant Sci 8:1881

Patil M, Ramu S, Jathish P, Sreevathsa R, Reddy PC, Prasad T, Udayakumar M (2014) Overexpression of AtNAC2 (ANAC092) in groundnut (*Arachis hypogaea* L.) improves abiotic stress tolerance. Plant Biotechnol Rep 8(2):161–169

Peng Z, Zhao Z, Clevenger JP, Chu Y, Paudel D, Ozias-Akins P, Wang J (2020) Comparison of SNP calling pipelines and NGS platforms to predict the genomic regions harboring candidate genes for nodulation in cultivated peanut. Front Genet 24:222

Prasad PVV, Craufurd PQ, Summerfield RJ (1999a) Sensitivity of peanut to timing of heat stress during reproductive development. Crop Sci 39:1352–1357

Prasad PVV, Craufurd PQ, Summerfield RJ (1999b) Fruit number in relation to pollen production and viability in groundnut exposed to short episodes of heat stress. Ann Bot 84:381–386

Prasad PVV, Craufurd PQ, Summerfield RJ, Wheeler TR (2000a) Effects of short episodes of heat stress on flower production and fruit-set of groundnut (*Arachis hypogaea* L.). J Exp Bot 51:777–784

Prasad PVV, Satyanarayana V, Potdar MV, Craufurd PQ (2000b) On-farm diagnosis and management of iron chlorosis in groundnut. J Plant Nutr 23:1471–1783

Prasad PVV, Craufurd PQ, Summerfield RJ (2000c) Effect of high air and soil temperature on dry matter production, pod yield and yield components of groundnut. Plant Soil 222:231–239

Prasad PVV, Boote KJ, Allen LH Jr, Thomas JMG (2003) Super-optimal temperatures are detrimental to reproductive processes and yield of peanut under both ambient and elevated carbon dioxide. Glob Change Biol 9:1775–1787

Prasad PVV, Boote KJ, Thomas JMG, Allen LH Jr, Gorbet DW (2006) Influence of soil temperature on seedling emergence and early growth of peanut cultivars in field conditions. J Agro Crop Sci 192(3):168–177

Prasad PVV, 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 PVV, Bheemanahalli R, Jagadish SVK (2017) Field crops and the fear of heat stress – opportunities, challenges and future directions. Field Crops Res 200:114–121

Prasad PVV, Craufurd PQ, Summerfield R (1999) Sensitivity of peanut to timing of heat stress during reproductive development. Crop Sci 39(5):1352–1357

Prasad PVV, Staggenborg SA, Ristic Z (2008b) Impact of drought and heat stress on physiological, growth and yield processes. In: Ahuja LH, Saseendran SA (eds) Modeling Water stress effects on plant growth processes. ASA – CSSA, Madison, WI, Vol 1, pp 301–355

Pruthvi V, Narasimhan R, Nataraja KN (2014) Simultaneous expression of abiotic stress responsive transcription factors, AtDREB2A, AtHB7 and AtABF3 improves salinity and drought tolerance in peanut (*Arachis hypogaea* L.). PLoS One 9(12):e111152

Qi Y, Wang H, Zou Y, Liu C, Liu Y, Wang Y, Zhang W (2011) Over-expression of mitochondrial heat shock protein 70 suppresses programmed cell death in rice. FEBS Lett 585(1):231–239

Qin H, Gu Q, Zhang J, Sun L, Kuppu S, Zhang Y, Burow M, Payton P, Blumwald E, Zhang H (2011) Regulated expression of an isopentenyltransferase gene (IPT) in peanut significantly improves drought tolerance and increases yield under field conditions. Plant Cell Physiol 52(11):1904–1914

Qin H, Feng S, Chen C, Guo Y, Knapp S, Culbreath A, He G, Wang ML, Zhang X, Holbrook CC (2012) An integrated genetic linkage map of cultivated peanut (*Arachis hypogaea* L.) constructed from two RIL populations. Theor Appl Genet 124(4):653–664

Quan X, Shan L, Bi Y (2007) Cloning of metallothionein genes from *Arachis hypogaea* and characterization of AhMT2a. Rus J Plant Physiol 54(5):669–675

Rami J-F, Leal-Bertioli SC, Foncéka D, Moretzsohn MC, Bertioli DJ (2014) Groundnut. In: Pratap A, Kumar J (eds) Alien gene transfer in crop plants, vol 2. Springer, pp 253–279

Ramu VS, Swetha TN, Sheela SH, Babitha CK, Rohini S, Reddy MK, Tuteja N, Reddy CP, Prasad TG, Udayakumar M (2016) Simultaneous expression of regulatory genes associated with specific drought-adaptive traits improves drought adaptation in peanut. Plant Biotechnol J 14(3):1008–1020

Rani KR, Chamundeswari K, Usha R (2018) Screening of thermotolerant groundnut genotypes using temperature induction response–a novel approach to assess genetic variability. Intl J Pharm Biol Sci 8:360–364

Rao RCN, Udayakumar M, Farquhar GD, Talwar HS, Prasad TG (1995) Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose-1, 5-bisphophate carboxylase in groundnut genotypes. Aust J Plant Physiol 22:545–551

Rao RCN, Talwar HS, Wright GC (2001) Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using a chlorophyll meter. J Agron Crop Sci 186:175–182

Ratnakumar P, Vadez V (2011) Groundnut (*Arachis hypogaea*) genotypes tolerant to intermittent drought maintain a high harvest index and have small leaf canopy under stress. Funct Plant Biol 38(12):1016–1023

Ravi K, Vadez V, Isobe S, Mir R, Guo Y, Nigam S, Gowda M, Radhakrishnan T, Bertioli D, Knapp S (2011) Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). Theor Appl Genet 122(6):1119–1132

Reddy T, Reddy V, Anbumozhi V (2003) Physiological responses of groundnut (*Arachis hypogea* L.) to drought stress and its amelioration: a critical review. Plant Growth Regul 41(1):75–88

Reddy K, Reddy K (1993) Genetic divergence in groundnut. Ann of Agric Res 14(1):9–14

Ren J, Wu P, Trampe B, Tian X, Lubberstedt T, Chen S (2017) Novel technologies in doubled haploid line development. Plant Biotechnol J 15:1361–1370

Ribaut J-M, Jiang C, Gonzalez-de-Leon D, Edmeades G, Hoisington D (1997) Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor Appl Genet 94(6–7):887–896

Ribaut J-M, Ragot M (2007) Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. J Exp Bot 58(2):351–360

Ruggiero A, Punzo P, Landi S, Costa A, Van Oosten MJ, Grillo S (2017) Improving plant water use efficiency through molecular genetics. Horticulturae 3(2):31

Sarkar T, Thankappan R, Kumar A, Mishra GP, Dobaria JR (2014) Heterologous expression of the AtDREB1A gene in transgenic peanut-conferred tolerance to drought and salinity stresses. PLoS One 9(12): e110507

Sarkar T, Thankappan R, Kumar A, Mishra GP, Dobaria JR (2016) Stress inducible expression of AtDREB1A transcription factor in transgenic peanut (*Arachis hypogaea* L.) conferred tolerance to soil-moisture deficit stress. Front Plant Sci 7:935

Sarvamangala C, Gowda M, Varshney R (2011) Identification of quantitative trait loci for protein content, oil content and oil quality for groundnut (*Arachis hypogaea* L.*).* Field Crops Res122 (1):49–59

Scott MF, Ladejobi O, Amer S, Bentley AR, Biernaskie J, Boden SA, Clark M, Dell'Acqua M, Dixon LE, Filippi CV (2020) Multi-parent populations in crops: a toolbox integrating genomics and genetic mapping with breeding. Heredity 125(6):396–416

Seijo G, Lavia GI, Fernández A, Krapovickas A, Ducasse DA, Bertioli DJ, Moscone EA (2007) Genomic relationships between the cultivated peanut (*Arachis hypogaea*, Leguminosae) and its close relatives revealed by double GISH. Amer J Bot 94(12):1963–1971

Selvaraj MG, Narayana M, Schubert AM, Ayers JL, Baring MR, Burow MD (2009) Identification of QTLs forpod and kernel traits in cultivated peanut by bulked segregant analysis. Electronic J Biotechnol 12(2):3–4

Selvaraj MG, Burow G, Burke JJ, Belamkar V, Puppala N, Burow MD (2011) Heat stress screening of peanut (*Arachis hypogaea* L.) seedlings for acquired thermotolerance. Plant Growth Regul 65(1):83–91

Shaibu AS, Sneller C, Motagi BN, Chepkoech J, Chepngetich M, Miko ZL, Isa AM, Ajeigbe HA, Mohammed SG (2020) Genome-wide detection of SNP markers associated with four physiological traits in groundnut (*Arachis hypogaea* L.) mini core collection. Agronomy 10(2):192

Shasidhar Y, Vishwakarma MK, Pandey MK, Janila P, Variath MT, Manohar SS, Nigam SN, Guo B, Varshney RK (2017) Molecular mapping of oil content and fatty acids using dense genetic maps in groundnut (*Arachis hypogaea* L.). Front Plant Sci 8:794

Shirasawa K, Koilkonda P, Aoki K, Hirakawa H, Tabata S, Watanabe M, Hasegawa M, Kiyoshima H, Suzuki S, Kuwata C (2012) In silico polymorphism analysis for the development of simple sequence repeat and transposon markers and construction of linkage map in cultivated peanut. BMC Plant Biol 12(1):80

Shirasawa K, Bertioli DJ, Varshney RK, Moretzsohn MC, Leal-Bertioli SC, Thudi M, Pandey MK, Rami J-F, Foncéka D, Gowda MV (2013) Integrated consensus map of cultivated peanut and wild relatives reveals structures of the A and B genomes of Arachis and divergence of the legume genomes. DNA Res 20(2):173–184

Shwetha, Sreenivasa AG, Ashoka J, Sushila N and Kuchnoor PH (2017) Effect of climate change on growth of groundnut (*Arachis hypogaea* L.). Intl J Pure Appl Biosci 5(6):985–989

Simpson C, Starr J, Church G, Burow M, Paterson A (2003) Registration of 'NemaTAM'peanut. Crop Sci 43(4):1561–1561

Singh A, Hariprassana K, Solanki R (2008) Screening and selection of groundnut genotypes for tolerance of soil salinity. Austral J Crop Sci 1(3):281–289

Singh N, Mishra A, Jha B (2014) Ectopic over-expression of peroxisomal ascorbate peroxidase (SbpAPX) gene confers salt stress tolerance in transgenic peanut (*Arachis hypogaea*). Gene 547(1):119–125

Singh A, Hariprasanna K, Chaudhari V (2016) Differential nutrients absorption an important tool for screening and identification of soil salinity tolerant peanut genotypes. Indian J Plant Physiol 21(1):83–92

Singh S, Abrol I (1985) Effect of soil sodicity on the growth, yield and chemical composition of groundnut (*Arachis hypogaea* Linn.). Plant Soil 84(1):123–127

Singh D, Singh SM (2000) Response of summer groundnut (*Arachis hypogaea*) and succeeding-maize (Zeamays) to sulphur and phosphorus fertilization. Indian J Agric Sci 70(10):657–60

Sinha P, Bajaj P, Pazhamala LT, Nayak SN, Pandey MK, Chitikineni A, Huai D, Khan AW, Desai A, Jiang H, Zhuang W, Guo B, Liao B, Varshney RK (2020) *Arachis hypogaea* gene expression atlas for fastigiata subspecies of cultivated groundnut to accelerate functional and translational genomics applications. Plant Biotechnol J 18(11):2187–2200

Songsri P, Jogloy S, Vorasoot N, Akkasaeng C, Patanothai A, Holbrook C (2008) Root distribution of drought-resistant peanut genotypes in response to drought. J Agro Crop Sci 194(2):92–103

Songsri P, Jogloy S, Holbrook C, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A (2009) Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agric Water Manage 96(5):790–798

Srivastava N, Vadez V, Narayan Nigam S, D Upadhyaya H, Narasu L (2018) Screening groundnut (*Arachis hypogaea* L.) germplasm for salinity tolerance. J Genet Res 4(2):130–140

Stalker HT (2017) Utilizing wild species for peanut improvement. Crop Sci 57(3):1102–1120

Stalker H, Tallury S, Ozias-Akins P, Bertioli D, Bertioli SL (2013) The value of diploid peanut relatives for breeding and genomics. Peanut Sci 40(2):70–88

Su L, Zhao C-Z, Bi Y-P, Wan S-B, Xia H, Wang X-J (2011) Isolation and expression analysis of LEA genes in peanut (*Arachis hypogaea* L.). J Biosci (Bangalore) 36(2):223–228

Subramanian V, Gurtu S, Rao RN, Nigam S (2000) Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. Genome 43(4):656–660

Sudhakar P, Kumar KV, Latha P, Sruthi VS, Sujatha K, Reddy BB, Reddy BR, Rajareddy K, Krishna TG, Reddy MS (2013) Efficacy of Pseudomonas fluorescens strains in enhancing drought tolerance and yield in peanut. In Recent advances in biofertilizers and biofungicides (PGPR) for sustainable agriculture. Proceedings of 3rd Asian Conference on Plant Growth-Promoting Rhizobacteria (PGPR) and other Microbials, Manila, Philippines, 21–24 April, 2013, pp. 268–274. Asian PGPR Society for Sustainable Agriculture, 2013

Sujay V, Gowda M, Pandey M, Bhat R, Khedikar Y, Nadaf H, Gautami B, Sarvamangala C, Lingaraju S, Radhakrishan T (2012) Quantitative trait locus analysis and construction of consensus genetic map for foliar disease resistance based on two recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). Mol Breed 30(2):773–788

Swamy BM, Upadhyaya H, Goudar PK, Kullaiswamy B, Singh S (2003) Phenotypic variation for agronomic characteristics in a groundnut core collection for Asia. Field Crops Res 84(3):359–371

Talwar H, Takeda H, Yashima S, Senboku T (1999) Growth and photosynthetic responses of groundnut genotypes to high temperature. Crop Sci 39(2):460–466

Tiwari V, Chaturvedi AK, Mishra A, Jha B (2015) Introgression of the SbASR-1 gene cloned from a halophyte Salicornia brachiata enhances salinity and drought endurance in transgenic groundnut (*Arachis hypogaea*) and acts as a transcription factor. PLoS One 10(7):e0131567

Tseng Y-C, Tillman BL, Peng Z, Wang J (2016) Identification of major QTLs underlying tomato spotted wilt virus resistance in peanut cultivar Florida-EP TM '113.' BMC Genet 17(1):1–14

Udayakumar M, Sheshshayee MS, Nataraj KN, Madhava HB, Devendra R, Hussain ISA, Prasad TG (1998) Why has breeding for water use efficiency not been successful? An analysis and alternate approach to exploit this trait for crop improvement. Curr Sci 74:994–1000

Upadhyaya HD (2005) Variability for drought resistance related traits in the mini core collection of peanut. Crop Sci 45(4):1432–1440

Upadhyaya HD, Nigam SN, Singh S (2001) Evaluation of groundnut core collection to identify sources of tolerance to low temperature at germination. Indian J Plant Genet Resour 14(2):165–167

Upadhyaya HD, Bramel PJ, Ortiz R, Singh S (2002) Developing a mini core of peanut for utilization of genetic resources. Crop Sci 42(6):2150–2156

Upadhyaya HD, Ortiz R, Bramel PJ, Singh S (2003) Development of a groundnut core collection using taxonomical, geographical and morphological descriptors. Genet Resour Crop Evol 50(2):139–148

Upadhyaya HD, Dwivedi SL, Nadaf HL, Singh S (2011) Phenotypic diversity and identification of wild Arachis accessions with useful agronomic and nutritional traits. Euphytica 182(1):103

Upadhyaya H, Dwivedi S, Vadez V, Hamidou F, Singh S, Varshney R, Liao B (2014) Multiple resistant and nutritionally dense germplasm identified from mini core collection in peanut. Crop Sci 54(2):679–693

Upadhyaya H, Reddy L, Dwivedi S, Gowda C, Singh S (2009) Phenotypic diversity in cold-tolerant peanut (*Arachis hypogaea* L.) germplasm. Euphytica 165(2):279–291

Vadez V, Rao S, Sharma KK, Bhatnagar-Mathur P, Devi MJ (2007) DREB1A allows for more water uptake in groundnut by a large modification in the root/shoot ratio underwater deficit. J SAT Agric Res 5:1–5

Vadez V, Rao JS, Bhatnagar-Mathur P, Sharma K (2013) DREB1A promotes root development in deep soil layers and increases water extraction under water stress in groundnut. Plant Biol 15(1):45–52

Valls JF, Simpson CE (2005) New species of Arachis (leguminosae) from Brazil, Paraguay and Bolivia. Bonplandia 14:35–63

Varshney RK, Mohan SM, Gaur PM, Gangarao N, Pandey MK, Bohra A, Sawargaonkar SL, Chitikineni A, Kimurto PK, Janila P (2013) Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnol Adv 31(8):1120–1134

Varshney RK, Thudi M, Pandey MK, Tardieu F, Ojiewo C, Vadez V, Whitbread AM, Siddique KH, Nguyen HT, Carberry PS (2018) Accelerating genetic gains in legumes for the development of prosperous smallholder agriculture: integrating genomics, phenotyping, systems modelling and agronomy. J Exp Bot 69(13):3293–3312

Varshney RK, Sinha P, Singh VK, Kumar A, Zhang Q, Bennetzen JL (2020) 5Gs for crop genetic improvement. Curr Opin Plant Biol 56:190–196

Varshney RK, Bertioli DJ, Moretzsohn MC, Vadez V, Krishnamurthy L, Aruna R, Nigam SN, Moss BJ, Seetha K, Ravi K (2009) The first SSR-based genetic linkage map for cultivated groundnut (*Arachis hypogaea* L.). Theor Appl Genet 118(4):729–739

Vishwakarma MK, Pandey MK, Shasidhar Y, Manohar SS, Nagesh P, Janila P, Varshney RK (2016) Identification of two major quantitative trait locus for fresh seed dormancy using the diversity arrays technology and diversity arrays technology-seq based genetic map in Spanish-type peanuts. Plant Breed 135(3):367–375

Wang ML, Sukumaran S, Barkley NA, Chen Z, Chen CY, Guo B, Pittman RN, Stalker HT, Holbrook CC, Pederson GA (2011) Population structure and marker–trait association analysis of the US peanut (*Arachis hypogaea* L.) mini-core collection. Theor Appl Genet 123(8):1307–1317

Wang H, Penmetsa RV, Yuan M, Gong L, Zhao Y, Guo B, Farmer AD, Rosen BD, Gao J, Isobe S (2012) Development and characterization of BAC-end sequence derived SSRs, and their incorporation into a new higher density genetic map for cultivated peanut (*Arachis hypogaea* L.). BMC Plant Biol 12(1):1–11

Wani SH, Sah SK, Sanghera G, Hussain W, Singh N (2016) Genetic engineering for cold stress tolerance in crop plants. Adv Genome Sci 4:173–201

Wilson JN, Chopra R, Baring MR, Selvaraj MG, Simpson CE, Chagoya J, Burow MD (2017) Advanced backcross quantitative trait loci (QTL) analysis of oil concentration and oil quality traits in peanut (*Arachis hypogaea* L.). Trop Plant Biol 10(1):1–17

Wynne J (1976) Use of accelerated generation increase programs in peanut breeding. In: Proceedings of American Peanut Research and Education Association, Inc., Vol 8, pp 44–47

Yang H, Nairn J, Ozias-Akins P (2003) Transformation of peanut using a modified bacterial mercuric ion reductase gene driven by an actin promoter from *Arabidopsis thaliana*. J Plant Physiol 160(8):945–952

Zhang H, Li G, Fu C, Duan S, Hu D, Guo X (2020) Genome-wide identification, transcriptome analysis and alternative splicing events of Hsf family genes in maize. Sci Rep 10(1):1–11

Zhao C, Qiu J, Agarwal G, Wang J, Ren X, Xia H, Guo B, Ma C, Wan S, Bertioli DJ (2017) Genome-wide discovery of microsatellite markers from diploid progenitor species, Arachis duranensis and A. ipaensis, and their application in cultivated peanut (*Arachis hypogaea*). Front Plant Sci 8:1209

Zhou X, Xia Y, Ren X, Chen Y, Huang L, Huang S, Liao B, Lei Y, Yan L, Jiang H (2014) Construction of a SNP-based genetic linkage map in cultivated peanut based on large scale marker development using next-generation double-digest restriction-site-associated DNA sequencing (ddRADseq). BMC Genomics 15(1):351

Zhou X, Xia Y, Liao J, Liu K, Li Q, Dong Y, Ren X, Chen Y, Huang L, Liao B (2016) Quantitative trait locus analysis of late leaf spot resistance and plant-type-related traits in cultivated peanut (*Arachis hypogaea* L.) under multi-environments. PLoS One 11(11):e0166873

Zhuang W, Chen H, Yang M, Wang J, Pandey MK, Zhang C, Chang W-C, Zhang L, Zhang X, Tang R (2019) The genome of cultivated peanut provides insight into legume karyotypes, polyploid evolution and crop domestication. Nat Genet 51(5):865–876

Zong H, Liu J, Wang F, Song N (2020) Root morphological response of six peanut cultivars to chromium (VI) toxicity. Environ Sci Pollut Res 27(15):18403–18411. https://doi.org/10.1007/s11356-020-08188-3

Chapter 5
Drought Tolerance in Rapeseed-Mustard: Conventional and Molecular Approaches

Maharaj Singh, V. V. Singh, Naveen Singh, and Monika

Abstract Rapeseed and mustard group of crops, cultivated in the arid and semi-arid regions of northern India, face many abiotic stresses especially drought, salinity, and high temperature. These stresses adversely affect plant growth and productivity. Among different abiotic stresses, drought stress is considered to be a major impediment in mustard cultivation. It is estimated that 40% of the world's land area is affected by drought. Approximately 55% of the agricultural lands in India are cultivated under rainfed situations, where mustard cultivation needs an alternative contingency plan. In genus *Brassica*, a significant inter and intra-specific variability exists for drought response, which needs to be exploited through genetic options. The presence of heritable variation in the gene pool of any crop is a prerequisite for defining a successful breeding program. Genetically diverse landraces, cultivated and/or wild relatives, evolved in different parts of the world, are the reservoirs of novel gene constellations required for better adaptation and performance under water deficit conditions. Availability of a relatively large number of crossable species in Brassicas and tissue culture mediated approaches provide ample options for incorporating better water productivity and drought tolerance in future varieties. Deployment of molecular markers and other biotechnological tools along with breeding approaches has the potential to improve precision, breeding efficiency and genetic gain through development of drought-tolerant varieties with higher productive. The correlated sub-traits and QTLs for responsive physiological traits imparting drought tolerance have already been identified by many workers. Therefore, it would be possible to transfer various drought-related traits into other adapted cultivars by involving molecular markers linked to identified QTLs. The transgenic approach, on the other hand, open-up new opportunities in the introgression of the genomic segment(s), which are responsible for enhancing the capacity of Brassicas to withstand drought, from

---

V. V. Singh (✉) · Monika
ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan 321303, India

M. Singh
ICAR-Indian Institute of Soybean Research, Indore, MP 452001, India

N. Singh
ICAR-Indian Institute of Agricultural Research, New Delhi, India

C. Kole (ed.), *Genomic Designing for Abiotic Stress Resistant Oilseed Crops*,
https://doi.org/10.1007/978-3-030-90044-1_5

unrelated species to cultivated ones. Advancement in terms of material and information and integration of different options and approaches shall help in mitigating the ill effects of limited water availability and improving rapeseed-mustard productivity in drought-affected areas.

Keywords Brassicas · Drought tolerance · Photosynthesis · Water use efficiency · Breeding · Marker-assisted selection · QTL mapping

5.1 Introduction

The crop plants face various abiotic stresses such as temperature (high/low), water stress (scarce/excess) and soil related salt/nutrient deficiencies and toxicities etc. The crop may also face one or more than one stresses at a time. High temperature coupled with drought, a very common phenomenon, adversely affect plant growth and productivity in the crop growing environments. Among these abiotic stresses, drought is the most serious problem which affects about 40% of the world's crop area and its population. Availability of insufficient fresh water for use is one of the curse to the mankind, which keep on challenging the habitat, livelihood, stability and sustainability of the living beings in the past. Drought occurs in regions consistently receiving a below-average or erratic rainfall accompanied by low atmospheric humidity. Drought can be broadly classified into (i) agricultural drought; (ii) meteorological drought; and (iii) hydrological drought. Agricultural drought is the most important and it occurs during periods of below-average precipitation and above-normal evaporation, thus, resulting in reduced plant growth and crop productivity. Limited water availability at the time of sowing reduce total area under cultivation in many parts of the world.

The genus Brassica harbours more than 100 species and most important among these are rapeseed (*Brassica napus* L.), mustard (*B. juncea* L.), cabbage (*B. oleracea* L.) and turnip rape (*B. rapa* L.). Species in this genus are being cultivated for oil, condiments, vegetables, or fodder purposes (Ashraf and McNeilly 2004). Rapeseed is the main oilseed crop of Europe and North America, while mustard is mainly cultivated in India, South East Asia and North Africa. Brassicas have a preference for different agro-ecological conditions, thus, have the potential to grow in the traditional as well as non-traditional areas with improved and well adapted varieties with matching management practices. Stability of performance in these conditions are always challenged by scarcity of irrigation water or untimely rainfall in rainfed areas. Agro-techniques developed for these regions create a responsive environment to realize the potential of the cultivars. Rapeseed mustard is an input responsive crop and has wider plasticity to different climates.

India is producing more than nine million metric tons of rapeseed-mustard seeds primarily from marginal lands of highly diverse environments by following agro-ecology specific varieties and cultivation practices. Fifty-five percent of the agricultural lands are rainfed, where cultivation of mustard can be explored with alternative contingency plan. Rapeseed-mustard due to its low water requirement (80–240 mm) fits well in the rainfed cropping systems of the country where crop is sown under conserved soil moisture. Although this group of crops possess better tolerance to drought than most of other crops, however, growing environments still demands a better level of tolerance. Significant inter-specific variation for drought tolerance exists within Brassica, which needs to be exploited through selection and breeding. Furthermore, wild and related species are reservoir of many useful agronomic traits including resistance to drought conditions. Such germplasm are potential sources for incorporating drought resistance in cultivated species (Warwick 1993).

5.2 Physiological Traits: Response to Drought

Drought is the most severe water stress that causes significant reduction in the growth and productivity of crop plants (Ludlow and Muchow 1990). Several physiological and biochemical processes are being altered by drought stress. Some of these alterations in the plants triggers adaptation mechanisms to tolerate drought stress. Degree of adaptation, enforced by decreased water potential, under drought condition varies among the species (Save et al. 1995). Drought causes morphological and biochemical changes in plants. Under severe conditions it losses plant parts and, thus, causing functional damage (Sangtarash 2010). Various physiological responses of plants with their tolerance mechanisms, such as pigment content and stability and high relative water content are known to determine ability to withstand drought (Clarke and McCaig 1982). The change in ratio of chlorophyll ‘a’ and ‘b’ and carotenoids content reported to be changed with onset of drought (Anjum et al. 2003 and Farooq et al. 2009). The response to drought stress is a function of genotype, intensity of stress and its duration, weather and growth conditions, and developmental stage of rapeseed (Robertson and Holland 2004).

5.2.1 Germination and Seedling Growth

Drought delay seed germination, reduces seedling vigour and, consequently poor seedling establishment. Seed germination under drought conditions is mainly affected by osmotic stress attributed directed to lower water absorption through the seed coat and, hence, reduced water uptake by the seeds under stress situations (Bahrami et al. 2012). Poor germination under water stress due to slower hydrolysis of storage compounds in endosperm or cotyledons and slower rate of transfer of hydrolysed material to the growing embryo axis was also reported (Ayaz et al. 2000). Further, the decrease in osmotic potential was identified to be main cause in lowering rate of water absorption, which results in decrease turgidity, cell division and finally decreased germination and growth (Zaefizadeh et al. 2011).

5.2.2 Phenology

During plant development, early vigour is an important trait under limited moisture conditions (Cairns et al. 2009). Faster phenology development under drought is a desirable trait and can alleviate the drought effects on yield. The degree of tolerance and trait variability is different from crop to crop. For example, seed size and early seedling vigour has been identified to be associated with drought tolerance in *B. juncea.* Seed size, in this species, was identified to be positively correlated with root length, root dry weight, root to shoot ratio and vigour index (Singh et al. 2012). It was also reported that genotypes with larger size results in improved shoot dry weight (13–43%), biomass (25–57%) and seed yield (12%) as compared to small seeds. Initial seedling weight, strongly correlated with seed size, emanates in higher shoot dry weight, biomass and yield (Elliott et al. 2017). Besides the effect of seed size on plant biomass and yield, larger seed size also ensures the tolerance to flea beetle in mustard. Hence, initial seedling growth and vigour are highly desirable characteristic of a cultivar, which affects plant growth and development under stress conditions.

Water stress drastically reduce the number of branches per plant, number of siliquae on the main shoot and the number of seeds per siliqua in drought-sensitive rapeseed genotypes (Zakirullah et al. 2000; Singh et al. 2002). Moreover, drought stress at the flowering stage considerably reduces the number of siliquae (Shirani and Daneshian 2006; Tribay and Renard 1999) and siliquae size, probably due to shortage of photosynthetic substances created by water stress (Rao and Mendham 1991). Water deficit stress during the flowering stage until the maturity in rapeseed cultivars cause a reduction in seed yield, biological yield, number of siliquae per plant, number of seeds per siliqua, 1000-seed weight, oil content, and oil yield (Nasri et al. 2008; Sinaki et al. 2007; Ali et al. 2009).

5.2.3 Root Architecture

Sufficient information on the role of root traits in drought avoidance among crop plants is available (Courtois et al. 2009; Maurel et al. 2010; Yamaguchi and Sharp 2010). Since higher root length and root mass facilitates water uptake under drought conditions, these are considered important trait for any drought-tolerant cultivar (Turner 1986). An increase in root depth in genotypes of different Brassicas was studied and it was reported that average root zone depth was 118.2 cm in *B. juncea* and 109 cm in *B. napus.* Higher root depth in *B. juncea* has lead to higher soil moisture extraction from deeper soil layers than that in *B. napus.* Root zone depth was positively correlated with the number of primary and secondary branches and the number of pods per plant in *B. juncea* while no such correlation was found in *B. napus* (Singh et al 2003; Singh and Kumar 2005). Increased water use in *B. juncea* through elevated plant water status (LWP and RWC) and photosynthetic activity helps in stabilizing productivity under water stress. Under drought conditions, a positive association was also observed between seed yield per plant and seedling root traits viz., root length, fresh and dry root weight (Cheema and Sadaqat 2004). Moderate level of stress is known to accelerate root growth through abscisic acid (ABA) synthesis.

Association of yield related traits with seedling root traits appeared to be more important than seedling shoot traits, under limited water conditions, established that roots as a more powerful sink. The root needs to grow longer for exploring deeper soil layers for water to drive nutrients and photo synthates for their development under limited water conditions. A well-developed and deep root system will have a higher capacity to absorb water and minerals and transport them to the growing shoots. Therefore, selection under water stress conditions, contrary to normal conditions, should be based on root traits rather than on shoot characteristics.

5.2.4 Plant Water Relation and Osmotic Adjustment (OA)

The physiological and biochemical changes in plants, under drought conditions, helps in their adaptation under stress by maintaining growth and productivity. Such established parameters could help in the screening and indirect selection of tolerant genotypes. Plant recovery from drought stress primarily depends on the capacity to maintain higher Relative Water Content (RWC) (Blum et al. 1999). A significant reduction in dry matter accumulation, chlorophyll content, RWC and, hence, seed and oil yield under rainfed conditions was also reported (Lallu 2012). Osmotic adjustment, on the other hand, plays an important role in maintaining turgor in cells under declining water potential. It also helps in maintaining stomatal conductance and photosynthesis at lower water potential. Turner et al. (2001) reported delayed leaf senescence and death, reduced flower abortion, improved root growth and increased water extraction from the soil due to increased osmotic potential. In general, water stress enforces osmotic dehydration of the plant tissues resulting in altered plant water relations. Variation in the maintenance of internal plant water status at the time of flowering was associated with grain yield under drought conditions (Blum 2002).

Leaf water potential (LWP) is an index for whole-plant water status (Turner 1982). Under the irrigated condition, transpiration in plants creates a negative LWP resulting into uptake of water from the soil. Whereas under drought conditions, highly negative LWP results in cavitation and create sturgor loss, and ultimately wilting of the plant.

Reduced relative water contents and osmotic potential, and increase in total greenness and proline contents were observed in mustard under different levels of water stresses (Alikhan et al. 2010). In *B. juncea*, water deficit decreases LWP and leaf RWC resulting in greater osmotic adjustment and higher root growth. Such adjustments help the plants to explore greater soil volume for water availability, thus, resulting in better yield attributes and seed yield. *B. juncea* had better osmotic adjustment than *B. napus* and a decrease in LWP, RWC and osmotic potential promote root growth in *B. juncea* better than in *B. napus* (Indo-Australia Final Project report 2009). Relative water content is closely related to cell size and determine the intricate relationship between transpiration and water supply to the leaf (Fischer and Wood 1979). The genotypes that maintain higher LWP and RWC are able to tolerate drought by maintaining higher water status (Kamoshita et al. 2008). The studies also suggested that differences for RWC among the genotypes, influenced by the severity of stress, adaptation and plant maturity, can be used as a secondary selection criterion (Lafitte 2002). In Brassicas, the differences for osmotic adjustment can be associated with production under drought stress (Kumar et al. 1984), however, such adjustments are growth stage dependent. In mustard, genotypes are known to articulate osmotic adjustment at anthesis but not at seed-filling stage (Ma et al. 2006).

5.2.5 Oxidative Damage

Reactive oxygen species (ROS) are synthesised in the plants as by-products of various intricate metabolic pathways occurred in different cellular compartments. The synthesis of ROS in the plants is a regular process occurring in chloroplasts, mitochondria and peroxisomes. Equilibrium between production and scavenging of ROS is largely affected by different abiotic stress including drought (Apel and Hirt 2004). Osmotic stress increases free proline, H_2O_2 malondialdehyde content (MDA) and lipoxygenase (LOX) activity in Brassicas (Alam et al. 2013). Variation in ascorbate content in different Brassica species has been reported. In *B. napus*, *B. campestris*, and *B. juncea* it decreased, increased, and remained unaltered, respectively, on the onset of water stress. As a consequence of osmotic stress, increase in glutathione (GSH) and glutathione S-transferase (GST) content and decreased activities of catalase (CAT) and mono dehydroascorbate reductase (MDHAR) was also reported in *B. juncea* (Alam et al. 2014). Furthermore, osmotic stress is responsible for increase in the glutathione disulfide (GSSG) content and decrease the GSH/GSSG ratio in all the *Brassica* species.

5.2.6 Stomatal Behaviour, Gas Exchange Parameters and Water Use Efficiency

Stomatal behaviour regulates both transpiration rate and net assimilation, which are crucial for improving crop water use efficiency (WUE). Regulation of stomata by guard cells determines the amount of CO_2 available for photosynthesis and the amount of water loss through transpiration (Lawson et al. 2014). Speed of stomatal response to the changed environment, stomatal density and mesophyll conductance to CO_2 have been proposed as important traits for adaptation under drought condition (Lawson and Blatt 2014; Franks et al. 2015). The stomatal response, and not the stomatal density, is more likely to enhance WUE (Lawson and Blatt 2014) and stomata get close with decreased LWP (Brodribb and Holbrook 2003). It has been observed that photosynthesis gets supressed when RWC falls around 70% (Lawlor and Cornic 2002). Therefore, the photosynthetic apparatus's resistance to water stress is also an important trigger for stomatal closure. Thus, drought reduces photosynthesis, transpiration, water use efficiency and seed yield. Furthermore, photosynthesis, transpiration, water use efficiency (WUE), stomatal conductance, leaf area index (LAI), specific leaf area (SLA), total dry matter (TDM) and seed yield were significantly affected by genotypes, environment and intricate relationship between them. In *B. juncea* genotypes, photosynthesis and water use efficiency are known to had a positive and significant correlation with seed yield under both irrigated rainfed conditions (Singh et al. 2009). Improving WUE would reduce the water requirement for realizing defined yield potential and, thus, help in saving a considerable amount of irrigation water. Using the carbon isotope discrimination technique, genotypic variation for WUE has been reported in peanut (Wright et al. 1988) and mustard (Singh et al. 2007). A positive and significant association of WUE with total dry matter and seed yield was also reported under the rainfed condition (Singh et al. 2009). Furthermore, genotypes with thicker leaves have observed greater WUE (Singh et al. 2003). Transpiration efficiency (TE) also influence the performance of crop under limited water availability. The ratio of photosynthesis rate to transpiration decreased when leaf vapour pressure deficit increase as a consequence of stress in rapeseed.

5.2.7 Canopy Temperature

Canopy temperature, directly related to stomatal conductance, is an important determinant of water stress tolerance. The high stomatal conductance favours more transpiration, thus, maintains the cooler canopy temperature. Canopy temperature and canopy temperature depression (CTD), under drought stress, are the reliable indicators of cooling of leaves under hot and humid climates achieved through transpiration.

On the onset of drought, stomata start closing, transpiration decreases and canopy temperature rises progressively. The close association of osmotic adjustment with both stomatal conductance and canopy temperature has been reported in different *Brassica* species by Kumar et al. (1984) and Singh et al. (1985). Genotypes with better osmotic adjustment maintained higher stomatal conductance and transpirational cooling (higher Tc-Ta) as compared to ones with low-osmotic adjustment. Drought tolerant *B. carinata*, in general, had cooler leaves as compared with *B. juncea, B. rapa* and *B. napus*. The differences in Tc-Ta were perhaps due to differences in stomatal conductance (Kumar and Singh 1998). Canopy temperature (CT) has, thus, established as screening technique for drought stress tolerance. With the advent of portable infrared thermometers (IRT) this approach has extensively being used for differentiating drought tolerance and susceptibility among genotypes. Water stress increases leaf temperature and decreases the difference between canopy and air temperature. Application of potassium decreased the transpirational losses, whereas, (Tc-Ta) increased irrespective of sampling stages and stress levels (Fanaei et al. 2009).

5.2.8 Photosynthesis, Physiological Parameters and Crop Productivity

Mustard genotypes carrying gene constellations responsible for governing water stress related traits/sub-traits imparts drought tolerance and observed little or no loss in performance under water deficit conditions. As we have already discussed, in this chapter, that drought is a complex phenomenon and response to which is determined by large number of morphological, physiological and biochemical sub-traits. In rapeseed-mustard, osmotic adjustment (Singh et al. 1996), transpirational cooling (Chaudhary et al. 1989), epicuticular wax on leaves, the difference between air and canopy temperature, DSI (Singh and Chaudhary 2003) can easily differentiate the tolerant and susceptible types. The physiological responses to drought stress may vary with developmental stages, and thus, make the screening difficult. The above mentioned traits are, therefore, shall be used for the phenotyping of drought tolerance (Tuberosa 2012). At present, measurable traits like water-use efficiency (WUE), drought susceptibility index (DSI), relative vigour index (RVI) and leaf wilting index (LWI), are widely used in breeding programmes directed towards development of drought tolerant cultivars in rapeseed-mustard (Tables 5.1 and 5.2).

Water potential, biomass and DSI can be used as criterion to evaluate the different Brassica species for relative drought tolerance (Ashraf and Mehmood 1990; Chaunhan et al. 2007). Specific leaf areas, on the other hand observe a significantly negative association with WUE, Total Dry Matter (TDM) and seed yield under both irrigated and rainfed conditions. A positive and significant association of photosynthesis with WUE, total dry matter and seed yield under rainfed conditions was reported by Singh and co-workers in 2009. The significantly positive association of WUE under the rainfed condition with TDM and seed yield. However, transpiration observed a significantly positive relationship with stomatal conductance and LAI under irrigated condition. Besides the plant phenological traits, water stress also known to cause increase in proline content, closure of stomata and inhibition of photosynthesis and hence reduced plant growth and development. Water stress also induced a significant difference in chlorophyll contents and accumulation of proline in *Brassica* species (Gibon et al. 2000). Associations of osmotic adjustment with stomatal conductance and canopy temperature have also been reported in Brassicas (Kumar et al. 1984; Singh et al. 1985).

Significant differences were observed among the various canola accessions for chlorophyll content and proline accumulation (Din et al. 2011). The reduction in chlorophyll content as a consequence of drought may be due to loss of pigment by disorganization of thylakoid membranes (Ladjal et al. 2000). This reduction in

Table 5.1 Traits at different developmental stage in different species under drought regime in *Brassica*

Traits or method	Species	Developmental stage	Traits	Drought regime	References
Seedling length, SSD, RVI	*B. napus*	Germination	Biological and biophysical traits	Petri-dish, 10% PEG-6000 solution	Yang et al. (2007)
Drought susceptibility Index (DSI)	*B. juncea*	Maturity	Seed yield and yield attributes	Experimental field	Chauhan et al. (2007)
Leaf wilting index (LWI)	*B. napus*	Seedling	Biological and biophysical traits	Pots in rain-out shelter	Li et al. (2012)
Principal component, clustering, subordinate function analysis	*B. napus*	Flowering	Morphological and agronomic traits	Rain-out shelter	Zhu et al. (2011)
Total dry matter (TDM), LAI, RGR, CGR	*B. napus*	Whole stage	Physiological growth indices	Field	Moaveni et al. (2010)
Biomass and water potential	*B. napus, B. juncea, B. camperstris, B. carinata*	Seedling and vegetative	Biomass, water potential, osmotic potential	Green house	Ashraf and Mehmood (1990)

chlorophyll content under drought stress may be attributed to (i) reduction in synthesis of the main chlorophyll pigment complexes (Allakhverdiev et al. 2000), (ii) destruction of chiral macro-aggregates of light-harvesting chlorophyll "a" or "b" pigment-protein complexes (CHCIIs), which protect the photosynthetic apparatus and/or (iii) oxidative damage of chloroplast lipids, pigments and proteins (Tambussi et al. 2000; Guo et al. 2015). The Chla/Chlb ratio numerically increases under moderate stress while it decreases under severe stress. This is presumably due to faster reduction of Chla content compared to that of Chlb under moderate stress conditions. Overall, lot of efforts has been made in finding out and associating physiological sub-traits to drought tolerance in Brassicas. Such information shall be highly useful in indirect selection of genotypes/germplasm/populations more efficiently.

Table 5.2 Formula for calculation of different stress indices

Index	Formula
Seedling Survival after Drought (SSD)	Calculated in percent
Relative Vigor Index (RVI)	(Cs × Ds)/(Ci × Di), where Cs and Ci are lengths of seedlings under drought-stressed and irrigated conditions, respectively. Whereas, Ds and Di are seedling survival (%) under drought and irrigated conditions, respectively
Drought Susceptibility Index (DSI)	(1 − As/Ai)/(1 − Bs/Bi) where, As and Ai are traits for a given genotype measured under drought-stressed and irrigated conditions, respectively
Leaf wilting index (LWI)	(1 − E/F) × 100, where E is the number of wilted leaves and F is the total number of leaves
Total Dry Matter (TDM)	Total dry matter of 10 plants
Leaf Area Index (LAI)	One-side green leaf area per unit ground surface area in broadleaf canopies
Relative Growth Rate (RGR)	(1/W) (dW/dt) so that RGR is increasing in dry mass (dW) per increase in time (dt) divided by existing biomass (W)
Crop growth rate (CGR)	The crop growth rate is increase in mass over a period of time

5.3 Breeding for Drought Tolerance

Breeding oilseed Brassicas for tolerance to drought is necessary to develop the cultivars with enhanced and sustained production under rainfed conditions. The crop genotypes must be screened under target environments having an adequate degree of stresses to quantify the sources of variation. Since this approach is highly dependent of environmental fluctuations, therefore, chances of failure of experiments is always there. Furthermore, mimicking the natural environmental conditions under artificial screening is very difficult and cost in-effective. On the other hand, plant responses to water stress are influenced by time, duration, frequency and intensity of stress. Plant, soil and climatic conditions are continuously interacting for determining the response of plants to water deficient conditions. It is difficult to establishment of well-defined and repeatable water stress conditions, thus, making screening of drought-tolerant genotypes more difficult (Ramirez and Kelly 1998). Therefore, different selection indicators should be used for the phenotyping of drought tolerance (Tuberosa 2012). A number of traits such as leaf wilting index (LWI), water-use efficiency (WUE), stress tolerance index (STI), drought susceptibility index (DSI) and relative vigor index (RVI) are widely used in breeding programmes for the identification of genotypes which produce higher yield under stress conditions.

Drought tolerance is a complex of traits controlled by relatively large number of genes (Blum 2005). Magnitude of heritable variation, for any quantitative traits, in the gene pool of a crop is prerequisite for any genetic improvement. Genetic diversity is already available in form of well-adapted cultivars/improved germplasm, landraces, relatives and wild species. Selection for drought tolerance is associated with early flowering and partitioning of dry matter to reproductive parts. Of course, selection for earliness is difficult due to its continued fruit-bearing behaviour till maturity. Dry-matter partitioning in reproductive parts is more prominent in rainfed than in irrigated condition. Anthesis, harvest index and maturity, on the other hand, are largely influenced by drought stress and, thus, affects screening of tolerant genotype. Biomass production and its partitioning, known to influence yield, is a result of intricate relationship among hundreds of genes and their interactions with environment. Rapeseed-mustard genotypes in which pods bearing and their maturity begins early in the season are considered to be more desirable for cultivation in stress prone areas. The phenomena of drought escape and drought avoidance are also operative in Brassicas and a number of mustard varieties viz., RH 725, DRMR 1165-40, DRMR 150-35, Aravali, Geeta, GM-1, PBR 97, Pusa Bahar, Pusa Bold, RH 781, RH 819, RGN 48, RB 50, Shivani, TM-2, TM-4 and Vaibhav are released for drought affected areas of India.

5.4 Genes Governing Drought Tolerance

Continuous efforts are being made to elucidate the molecular mechanism of drought tolerance in plants. A total of 1092 drought-responsive genes have been discovered, of which 37 are transcription factors. Out of these drought-responsive genes, 28 were related to signal transduction and 61 were from osmo-sensing-responsive pathways. Three hundred and eight down-regulated and 248 up-regulated genes were also reported for drought in *Sinapis alba* (Dong et al. 2012). Gene ontology (GO) analysis showed differentially expressed genes involved in cell division, catalytic and metabolic processes. Many of these genes were involved in response to abscisic acid (ABA) or water stress, indicating that ABA and water stress-mediated signal transductions are the probable mechanisms for the root hydrotropic response. With the availability of a large number of candidate genes imparting drought tolerance, genetic enhancement for drought tolerance in Brassicas can be achieved through manipulating their expression. Relatedness of this species to *Arabidopsis* shall be further helpful in predicting response of these genes.

With the open access to the massive gene expression data and bioinformatic tool for predicting key genes involved in water stress genes were confirmed to related to known biological processes involved in imparting resistance to drought (Liang et al. 2011). A number of genes (about 500) were identified to be linked to the stress response and the ABA response (Liang et al. 2011). In another attempt, overexpression of an ethylene-responsive factor (ERF) from *B. rapa* (BrERF4) increased *Arabidopsis* resistance to salt and drought stresses. BrERF4 expression was triggered by ethylene or methyl jasmonate but not by ABA or NaCl (Seo et al. 2010), thus, suggesting that BrERF4 seems to be activated through a network of diverse signaling pathways in response to these two stresses. *Arabidopsis* lateral suppressor (LAS) homologous gene (BnLAS) from *B. napus* was cloned and found to be expressing in the roots, shoot tips, lateral meristems, and flower organs of the plants (Yang et al. 2011). Annexin gene (AnnBn1), isolated and cloned from the drought-tolerant rapeseed genotype 'Q2' (Xiao et al. 2012), encoding Annexins in genus *Brassica* play an important role in abiotic stress responses (Jami et al. 2008). With the availability of tools to design gene based markers and highly efficient and reproducible molecular markers, these identified genes can be edited or staked into improved genetic backgrounds.

5.5 Deployment of QTLs Through Marker Assisted Selection

Although conventional breeding was successful in the past century in raising the yield potential of the crop (Campos et al. 2004; Borlaug and Dowswell 2005; Duvick 2005), however, efforts in development of drought tolerant varieties are sporadic and largely unsuccessful. At this stage, plant breeders have either little or no knowledge of the factor responsible for genetic variability for response to drought (Blum 1988; Borlaug 2007). Little/no information on variability, inheritance and genome segment governing the trait and linked molecular markers for sub-traits governing drought tolerance was the major impediment in transferring the drought tolerance in improved genetic backgrounds. Thus, continuous efforts were made to understand or elucidate genetic bases of phonological characteristics for e.g. stay green trait (Jiang et al. 2004; Verma et al. 2004); genetic variation for osmotic adjustment (Teulat et al. 1998; Robin et al. 2003); root growth rate, length and biomass in exploiting soil moisture (Johnson et al. 2000; Nguyen et al. 2004); reduction in leaf area and reduction in growth period (Anyia and Herzog 2004); limitation of non-stomatal water loss from leaves (Lafitte and Courtois 2002), and response of leaf elongation rate to soil moisture (Reymond et al. 2003).

Since conventional approaches are not efficient and demands improvement considering the increasing frequency and severity of drought imposed by continuously changing climate. For improving sustainability and stability of yield under stress conditions, the breeder has to adopt cost and time efficient approaches in breeding programs where the genetic dissection of the quantitative traits is a prerequisite for controlling the adaptive response of crops to abiotic stress. Response to most of abiotic stresses is quantitative in nature and are genetically determined by Quantitative Trait Loci (QTLs). QTL analysis provided unprecedented opportunities in finding out chromosome regions that controls variations for different morphological, physiological, and biochemical changes affecting plant growth under water-stressed environments. The QTL approach is considered to be a more logical approach that helps to reveal the genetic as well as physiological components distressing source-sink relationships under abiotic stress (Miralles and Slafer 2007; Welcker et al. 2007). Molecular markers led identification of QTLs has helped in establishing their linkage with physiological sub-traits imparting drought tolerance (Chinnusamy et al. 2005; Hussain 2006). Once the QTLs linked to specific sub-traits for drought tolerance are tagged with molecular markers, their transfer to other genetic backgrounds through Marker-Assisted Selection (MAS) becomes possible.

Using 72 Double Haplid (DH) lines derived from a cross between drought and salinity tolerant genotype (TX9425) with a sensitive variety (Franklin) of Brassica. The QTLs responsible for leaf wilting under drought stress were identified on 2H and 5H chromosomes. The QTL located on chromosome 2H explained 42% of phenotypic variation, whereas, the one on chromosome 5H was less affected by agronomic backgrounds (Fan et al. 2015). Thirty QTLs for water use efficiency (WUE), indicated by carbon isotope ratios and photosynthetic traits, were mapped and significant QTLs on 7 linkage groups were reported to explain 3.4–36.6% of the phenotypic variance (Hall et al. 2005). Further, QTLs for several physiological characters like photosynthetic capacity, nitrogen content, leaf thickness and stomatal density were reported at the same location indicating that gene(s) at these loci may have pleiotropic effects for traits related to water-use and photosynthesis. The QTLs responsible to govern root pulling force trait had relatively small contributions to the phenotypic variation, although some of the QTLs were consistent across the years. In addition to this, a major QTL for days to flowering detected on linkage group 2. QTLs for plant height are mapped on LG 14 T, however, no stable QTLs for seed yield under stress is reported in genus *Brassica* (Mahmood et al. 2005). However, low constancy and reliability of identified QTLs was also reported using RIL populations developed using contrasting values for days to 50% flowering, length of root and root length reduction rate and percentage of fully emerged cotyledons. Furthermore, some QTLs for root length and response were found overlapped on chromosome 1 and 3 in *Arabidopsis thaliana.* Signifying that these two loci may contain genes which regulate root length and tolerance.

With the availability of molecule markers tightly linked to loci governing response to drought stress, application of Marker-assisted selection (MAS) has strength to precisely assemble them to any improved background with high precision. For achieving this, parents with extreme contrasting sub-traits, especially yield under stress and drought tolerance, need to be identified and mapped. QTLs linked to different drought related sub-traits has been identified, however, efforts for validation of these QTLs in altogether different genetic backgrounds and development of reliable and linked molecular marker system need to be directed for successful deployment of MAS in incorporation of drought tolerance. In this endeavour, the approach of QTL mapping has become crucial to the use of DNA markers in the improvement of crops species (Ramchiary et al. 2007). MAS potentially helps in shortening the cycle of selection and improving genetic gains (Moose and Mumm 2008).

Marker-assisted selection is non-destructive, efficient, reliable and stage independent method used successfully with identified DNA markers that flank a gene of interest or any segment of DNA. This approach can be used to gain information on the genotype of a single plant or a large number of samples at a time without exposing the plant to stress. This technique provides an authoritative tool to shorten breeding cycles conducted for enhancement of plant stress tolerance. In past two decades, development of molecular markers linked to physiological traits has made significant headway and is considered as a major breakthrough in crop improvement. Use of molecular markers has increased rapidly and lead to the development of detailed molecular linkage maps for many plant species (Jain and Selvaraj 1997), including rapeseed-mustard (Yangj et al. 2016). Different types of DNA markers such as AFLP, RFLP, SSR and RAPD are being using in rapeseed-mustard breeding programmes for mapping, tracking of genes/QTLs, trait discovery and diversity analysis etc. (Saranga et al. 2001; Lionneton et al. 2002; Sharma et al. 2002; Pradhan et al. 2003; Quesada et al. 2002; Ramchiary et al. 2007; Vinu et al. 2013; Thakur et al. 2018). Various markers such as random amplified polymorphic DNA, restriction fragment length polymorphism, amplified fragment length polymorphism and simple sequence repeat analyses have been used in the marker-assisted selection in various crops of genus *Brassica.* With the available information and material, it has now become possible to integrate MAS actively in breeding programmes directed towards development of high yielding stress tolerant, including drought tolerant, rapeseed-mustard cultivars for fragile environments.

5.6 Conclusions and Future Perspective

Most of *Brassica* cultivars do not perform satisfactorily under field stress conditions due to their sensitivity to drought stress. Physiological and phenotypic traits associated with drought-tolerant serve as important criterion for identifying stress-tolerant genotypes and introducing tolerance into cultivated genotypes. Efforts were made in the past to utilize the intra-specific variability, however, only limited success is achieved. Alternatively, high degree of drought tolerance has been identified in wild and weedy relatives, however, their transfer to cultivated germplasm has not been much successful due to poor fertility in the progenies generated through sexual hybridization and severe linkage drag. With the availability of tissue culture procedures and protocols and information on location of responsive gene/QTLs and available linked molecular markers, it is now possible to synthesise the fertile progenies and tag the genomic segments from wild resources. Molecular markers have strength to faster identification of genes or QTLs associated with drought stress tolerance and their deployment. Furthermore, the recombinant DNA approach promises to introduce traits from unrelated sources. Engineered genes encoding osmolytes, plant growth regulators, late embryogenesis abundant proteins, antioxidants, and transcription factors introduced into transgenic lines performed well under controlled stress conditions. However, practical implication of this could not be fully encouraged and utilized due to regulatory and technical issues. Further, with the discovery of new cis-genic approaches like CRISPR-(clustered regularly interspaced short palindromic repeats)-Cas gene editing, to modify the function of inhabited gene(s) has become possible. Use of CRISPR (clustered regularly interspaced short palindromic repeats)-Cas technology, an effective, novel and holistic approach, shall open up new vistas for better understanding about the intricate relationship between drought related sub-traits and physiological and biochemical functions within and between different abiotic stresses. In future, this approach has potential to regulate and mitigate the ill effects of drought. With the advancement in science and technological breakthroughs it has now become possible to deploy multiple stress tolerance mechanisms in Brassicas to achieve high levels of tolerance, and thus, stabilize the performance and improve productivity in stress prone areas.

References

Alam A, Hasanuzzaman M, Nahar K, Fujita M (2013) Exogenous salicylicacid ameliorates short-term drought stress in mustard (Brassica juncea L.) seedlings by up-regulating the antioxidant defense and glyoxalase system. Aust J Crop Sci 7(7):1053–1063

Alam MM, Nahar K, Hasanuzzaman M, Fujita M (2014) Alleviation of osmotic stress in *Brassica napus*, *B. campestris*, and *B. juncea* by ascorbic acid application. Biol Plant 58(4):697–708

Ali Q, Ashraf M, Anwar F (2009) Physico-chemical attributes of seed oil from drought-stressed sunflower (Helianthus annuus L.) plants. Grasasy Aceites 60:475–548

Alikhan M, Ashraf MY, Mujtaba SM, Shirazi MU, Khan MA, Shereen A, Mumtaz S, Siddiqui MA, Kaleri GM (2010) Evaluation of high yielding canola type *Brassica*genotypes/mutants for drought tolerance using physiological indices as screening tool. Pak J Bot 42:3807–3816

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Murata N (2000) Inactivation of photosystems I and in response to osmotic stress in Synechococcus. Contribution of Water channels. Plant Physiol 122:1201–1208

Anjum F, Yaseen M, Rasul E, Wahid A, Anjum S (2003) Water stress in barley (*Hordeumvulgare*L.). II. Effect on chemical composition and chlorophyll contents. Pak J Agric Sci 40:45–49

AnyiaAO HH (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpea under mid-season drought. Eur J Agron 20:327–339

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Ann Rev Plant Biol 55:373–399

Ashraf M, McNeilly T (2004) Salinity tolerance in *Brassica* oilseeds. Crit Rev Plant Sci 23:157–174

Ashraf M, Mehmood S (1990) Response of four *Brassica* species to drought stress. EnvironExp Bot 30:93–100

Ayaz FA, KadiogluA URT (2000) Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthesetosa.* J Plant Sci 80:373–378

Bahrami H, Razmjoo J, Ostadi Jafari A (2012) Effect of drought stress on germination and seedling growth of sesame cultivars (*Sesamumindicum* L.). Int J AgriSci 2(5):423–428

Blum A (1988) Plant breeding for stress environments. CRC Press, Boca Raton, Florida

Blum A (2002) Drought tolerance is a complex trait. In: Saxena NP, O'Toole JC (eds) Field screening for drought tolerance incrop plants with emphasis of rice. ICRISAT, Patancheru, India, pp 17–22

Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Crop Pasture Sci 56(11):1159–1168

Blum A, Mayer J, Golan G, Sinmera B (1999) Drought tolerance of a doubled- haploid line population of rice in the field. In: Ito P, O'Toole JC, Hardy B (eds) Genetic improvement of rice for water limited environment. IRRI, Manila, Philippines, pp 319–330

Borlaug NE (2007) Sixty-two years of fighting hunger: personal recollections. Euphytica 157:287–297

Borlaug NE, Dowswell CR (2005) Feeding a world of ten billion people: a 21st-centurychallenge. In: Tuberosa R, Phillips RL, Gale M (eds) Proceedings of the international congress in the wake of the double Helix: from the green revolution to the gene revolution, 27–31 May 2003, Bologna, Italy. Avenue Media, Bologna, Italy, pp 3–23

Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. Plant Physiol 132:2166–2173

Cairns JE, Namuco OS, Torres R, Simborio FA, Courtois B, Aquino GA, Johnson DE (2009) Investigating early vigour in upland rice (*Oryzasativa* L.): part II. Identification of QTLs controlling early vigour under greenhouse and field conditions. Field Crops Res 113:207–217

Campos H, Cooper A, Habben JE, EdmeadesGO SJR (2004) Improving drought tolerance in maize: a view from industry. Field Crops Res 90:19–34

Chaudhary BD, Singh DP, Singh P, Kumar A (1989) Inheritance studies of plant water relations in *Brassica juncea.* BiolPlant 31:202–206

Chauhan JS, TyagiMK KA, Nashaat NI, Singh M, Singh NB, JakharML WSJ (2007) Drought effects on yield and its components in Indian mustard (*Brassicajuncea*L.). Plant Breed 126:399–402

Cheema KL, Sadaqat HA (2004) Potential and genetic basis of drought tolerance in Canola (*Brassica juncea*). V. Correlation studies between seedling and morphological traits under drought condition. J Agric Res 42(2):137–152

Chinnusamy V, Jagendorf A, Zhu JK (2005) Understanding and improving salt tolerance in plants. Crop Sci 45:437–448

Clarke J, McCaig T (1982) Evaluation of techniques for screening for drought resistance in wheat. J Crop Sci 22:503–506

Courtois B, Ahmadi N, Perin C, LuquetD GE (2009) The rice root system: from QTLs to genes to alleles. In: Serraj R et al (eds) Drought frontiers in rice: crop improvement for increased rainfed production. World Scientific Publishing, Singapore, pp 171–188

Din J, Khan SU, Ali I, Gurmani AR (2011) Physiological and agronomic response of canola varieties to drought stress. J Anim Plant Sci 21:78–82

Dong CH, Li C, Yan XH, Huang SM, Huang JY, Wang LJ, Guo RX, Lu GY, Zhang XK, Fang XP (2012) Gene expression profiling of Sinapis alba leaves under drought stress and re-watering growth conditions with Illumina deep sequencing. Mol Biol Rep 39:5851–5857

Duvick DN (2005) The contribution of breeding to yield advances in maize (Zea mays L.). Adv Agron 86:83–145

Elliott RH, Mann LW, Olfert OO (2017) Effects of seed size and seed weight on seedling establishment, seedling vigour and tolerance of summer turnip rape (*Brassica rapa*) to fleabeetles, Phyllotreta spp. Can J Plant Sci 385–393

Fan Y, Shabala S, Ma Y, Xu R, Zhou M (2015) Using QTL mapping to investigate the relationships between abiotic stress tolerance (drought and salinity) and agronomic and physiological traits. BMC Genomics 16:43

Fanaei HR, Galavi M, KafiM BAG (2009) Amelioration of water stress by potassium fertilizer in two oilseed species. Int J Plant Proc 3:41–54

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

Fischer R A, Wood JT (1979) Drought resistance in spring wheat cultivars III Yield association with morpho-physiological traits. Austral J Agric Res 30(6):1001–1020. https://doi.org/10.1071/AR9791001

Franks PJ, Doheny-Adams TW, Britton-Harper ZJ, Gray JE (2015) Increasing water-use efficiency directly through genetic manipulation of stomatal density. New Phytol 207:188–195. https://doi.org/10.1111/nph.13347 [PubMed] [Cross Ref]

Gibon Y, Sulpice R, Larher F (2000) Proline accumulation in canola leaf discs subjected to osmotic stress is related to the loss of chlorophylls and the decrease of mitochondrial activity. Physiol Plant 4:469–476

Guo Y, Abernathy B, Zeng Y, Ozias-Akins P (2015) TILLING by sequencing to identify induced mutations in stress resistance genes of peanut (*Arachishypogaea*). BMC Genomics 16(1): Article no. 157. Available at: https://doi.org/10.1186/s12864-015-1348-0

Hall NM, GriffithsH CJA, Jones HG, Lynn J, King GJ (2005) Relationships between water-use traits and photosynthesis in Brassica oleracea resolved by quantitative genetic analysis. Plant Breed 124:557–564

Hussain SS (2006) Molecular breeding for abiotic stress tolerance: drought perspective. Proc Pak Acad Sci 43(3):189–210

Jain RK, Selvaraj G (1997) Molecular genetic improvement of salt tolerance in plants. Biotechnol Annu Rev 3:245–267

Jami SK, Clark GB, Turlapati SA, Handley C, Roux SJ, Kirti PB (2008) Ectopic expression of an annexin from Brassica juncea confers tolerance to abiotic and biotic stress treatments in transgenic tobacco. Plant Physiol Biochem 46:1019–1030

Jiang GH, He YQ, Xu CG, Li XH, Zhang Q (2004) The genetic basis of stay-green in rice was analyzed in a population of doubled haploid lines derived from an indica by japonica cross. Theor Appl Genet 108:688–698

Johnson WC, Jackson LE, Ochoa O, Van Wijk R, Peleman J, St. Clair DA, Michelmore RW (2000) A shallow-rooted crop and its wild progenitor differ at loci determining root architecture and deep soil water extraction. Theor Appl Genet 101:1066–1073

Kamoshita A, Babu RC, BoopathiNM FS (2008) Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field Crops Res 109:1–23

Kumar A, Singh DP (1998) Use of physiological indices as a screening technique for drought tolerance in oilseed *Brassica* species. Ann Bot 81:413–420

Kumar A, Singh P, Singh DP, Singh H, Sharma HC (1984) Differences in osmoregulation in Brassica species. Ann Bot 54:537–541

Ladjal M, Epron D, Ducrey M (2000) Effects of drought preconditioning on thermotolerance of photosystem II and susceptibility of photosynthesis to heat stress in cedar seedlings. Tree Physiol 20:1235–1241

Lafitte HR, Courtois B (2002) Interpreting cultivar environment interactions for yield in upland rice assigning value to drought-adaptive traits. Crop Sci 42:1409–1420

Lafitte R (2002) Relationship between leaf relative water content during reproductive stage water deficit and grain formation in rice. Field Crops Res 76:165–174

Lallu KM (2012) Variability in biochemical and physiological parameters of mustard [*Brassica juncea* (L.) Czern & Coss] genotypes under rainfed and irrigated condition. Indian J Agric Biochem 25:129–137

LawlorDW CG (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ 25:275–294

Lawson T, Blatt M (2014) Stomatal size, speed and responsiveness impact on photosynthesis and water use efficiency. Plant Physiol 164:1556–1570. https://doi.org/10.1104/pp.114.237107

Lawson T, Simkin AJ, Kelly G, Granot D (2014) Mesophyll photosynthesis and guard cell metabolism impacts on stomatal behaviour. New Phytol 203:1064–1081. https://doi.org/10.1111/nph.12945

Li Z, Wu BJ, Lu GY, Chen Y, Zou CS, Zhang XK (2012) Differences in physiological responses of *Brassica napus* genotypes under water stress during the seedling stage. Chin J Oil CropsSci 34:033–039

Liang Y, Zhang F, Wang J, Joshi T, Wang Y, Xu D (2011) Prediction of drought-resistant genes in *Arabidopsis thaliana* using SVM-RFE. PLoS One 6:e21750

Lionneton E, Ravera S, Sanchez L, Aubert G, DelourmeR OS (2002) Development of an AFLP based linkage map and localisation of QTLs for seed fatty acid content in condiment mustard (*Brassica juncea*). Genome 45:1203–1215

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Advan Agron 43:107–153

Ma Q, Niknam SR, Turner DW (2006) Responses of osmotic adjustment and seed yield of *Brassica napus* and *B. juncea* to soil water deficit at different growth stages. Crop Pasture Sci 57:221–226

Mahmood S, Hassan S, Ahmed F, Ashraf M, Alam M, Muzaffar A(2005) Influence of feed withdrawal for different durations on the performance of broilers in summer. Int J Agri Biol 7:975–978

Maurel C, Simonneau T, Sutka M (2010) The significance of roots as hydraulic rheostats. J Exp Bot 61:3191–3198

Miralles DJ, Slafer GA (2007) Sink limitations to yield in wheat: how could it be reduced? J Agric Sci 145:139–149

Moaveni P, Ebrahimi A, Farahani HA (2010) Physiological growth indices in winter rapeseed (*Brassica napus*L.) cultivars as affected by drought stress at Iran. J Cereals Oilseeds 1:11–16

Moose SP, Mumm RH (2008) Molecular plant breeding as the foundation for 21st-century crop improvement. Plant Physiol 147:969–977

Nasri M, Khalatbari M, Zahedi H, Paknejad F, Tohidi-Moghadam HR (2008) Evaluation of micro and macro elements in drought stress condition in cultivars of rapeseed (Brassica napus L.) Amer J Agril Biol Sci 3:579–583

Nguyen TT, Klueva N, Chamareck V, Aarti A, Magpantay G, Millena AC, Pathan MS, Nguyen HT (2004) Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. Mol Genetgenom 272:35–46

Pradhan AK, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D (2003)A high-density linkage map in *Brassica juncea* (Indian mustard) using AFLP and RFLP markers. Theor Appl Genet 106:607–614

Quesada V, Garcia MS, Piqueras P, Ponce MR, Micol JL (2002) Genetic architecture of NaCl tolerance in Arabidopsis. Plant Physiol 130:951–963

Ramchiary N, Padmaja KL, Sharma S, Gupta V, Sodhi YS, Mukhopadhyay A, Arumugam N, Pental D, Pradhan AK (2007) Mapping of yield influencing QTL in *Brassicajuncea*: implications for breeding of a major oilseed crop of dryland areas. Theor Appl Genet 115:807–817

Ramirez P, Kelly JD (1998) Traits related to drought resistance in common bean. Euphytica 99:127–136

Rao MSS, Mendham NJ (1991) Soil plant water relations of oilseeds rape (*Brassica napus*) and (*B.campestris*). J Agric Sci Camb 117:197–225

Reymond M, Muller B, Leopardi A, Charcosset A, Tardieu F (2003) Combining quantitative trait loci analysis and an eco-physiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. Plant Physiol 131:664–675

Robertson MJ, Holland JF (2004) Production risk of canola in the semi-arid subtropics of Australia. Aust J Agril Res 55:525–538

Robin S, Pathan MS, Courtois B, Lafitte R, Carandang S, Lanceras S, AmanteM NHT, Li Z (2003) Mapping osmotic adjustment in an advanced back-cross inbred population of rice. Theor Appl Genet 107:1288–1296

Sangtarash MH (2010) Responses of different wheat genotypes to drought stress applied at different growth stages. Pak J Biol Sci 13:114–119

Saranga Y, Menz M, Jiang C, Wright R, Yakir D, Paterson AH (2001) Genomic dissection of genotype x environment adaptation conferring adaptation of cotton to arid conditions. Genome Res 11:1988–1995

Save R, Biel C, Domingo R, Ruiz-Sanchez MC, Torrecillas A (1995) Some Sci 110:167–172

Seo YJ Park JB, Cho YJ Jung C, Seo HS, Park SK Nahm BH, Song JT (2010) Overexpression of the ethylene-responsive factor gene BrERF4 from *Brassica rapa* increases tolerance to salt and drought in *Arabidopsis* plants. Mol Cells 30:271–277

Sharma R, Aggarwal RAK, Kumar R, Mohapatra T, Sharma RP (2002) Construction of an RAPD linkage map and localization of QTLs for oleic acid level using recombinant inbreds in mustard (*Brassica juncea*). Genome 45:467–472

Shirani Rad AH, Daneshian J (2006) Study of drought stress at different developmental stages on rapeseed cultivar. The Society for Conservation and Protection of Environment

Sinaki JM, MajidiHeravan E, Shirani Rad AH, Nour Mohammadi G, Zarei H (2007) The effects of water deficit during growth stages of canola (*B. napus* L.). Amer-Euras J Agril Environ Sci 2:417–422

Singh SP, Choudhary AK (2003) Selection criteria for drought tolerance in Indian mustard (Brassica juncea L). Indian J Genet 63:263–264

Singh M, Kumar A (2005) Changes in the physiological and biochemical characters of mustard genotypes under irrigated and rainfed condition. Oilseeds Res 22:235–237

Singh DP, Singh P, Kumar A, Sharma HC (1985) Transpirational cooling as a screening technique for drought tolerance in oilseed brassicas. Ann Bot 56:815–820

Singh DP, Sangwan VP, Pannu RK, Choudhary BD (1996) Comparison of osmotic adjustment in leaves and siliquae of oilseed brassicae. Indian JPl Physiol 1(4):284–285

Singh MP, Pandey UN, Lal RK, Chaturvedi GS (2002) Response of *Brassica* species to different irrigation regimes. Indian J Plant Physiol 1:66–69

Singh M, Chauhan JS, Kumar A, Singh NB (2003) Nitrogen assimilatory enzymes, chlorophyll content and yield as influenced by drought stress in Indian mustard (*Brassica juncea*L.). Brassica 5:42–47

Singh M, Chauhan JS, Meena SS (2009) Drought-induced changes in water use efficiency and other morpho-physiological characters in Indian mustard (*Brassica juncea*L.) 16th Australian Research Assembly on Brassicas. Ballarat Victoria 2009

Singh M, Chauhan JS, Sheshshyee MS, Udaya Kumar M, Kumar A (2007) Isotope discrimination technique (Δ13C): a possible selection criteria for drought tolerance in Indian mustard (*Brasicajuncea* L.). In: Proceedings of 12th International Rapeseed Congress, Wuhan, China

Singh M, Sharma A, Chauhan, JS, Meena RC, Meena ML, Meena SS, Mishra AP (2012) Influence of Seed Size on Germination and Early Seedling Growth in Indian Mustard(*Brassica juncea* L.). Indian J Plant Genet Resour 25(3):257–260

Tambussi EA, Bartoli CG, Bettran J, Guiamet JJ, Araus JC (2000) Oxidative damage to thylakoids proteins in water-stressed leaves of wheat (Triticumaestivum L.). Physiol Plant 108:398–404

Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, MonneveuxP CA (1998) Several QTLs involved in osmotic adjustment trait variation in barley (Hordeumvulgare L). Theor Appl Genet 96:688–698

Thakur AK, Singh KH, Singh L, Nanjundan J, Khan YJ, Singh D (2018) SSR marker variations in *Brassica* species provide insight into the origin and evolution of Brassicaamphi diploids. Hereditas 155(1):1–11

Tribay-Blondel AM, Renard M (1999) Effect of temperature and stress of fatty acid composition of rapeseed oil. In: Proceeding of the 10th international rapeseed congress, Australia

Tuberosa R (2012) Phenotyping for drought tolerance of crops in the genomics era. Front Physiol 3:347–356

Turner NC (1982) The role of shoot characteristics in drought tolerance of crop plants. In: Drought tolerance in crop with emphasis on rice. IRRI, Los Banos, Manila, pp 115–134

Turner NC (1986) Adaptation to water deficits: a changing perspective. Austral J Plant Physiol 13:175–190

Turner NC, Wright GC, Siddique KHM (2001) Adaptation of grain legumes (pulses) to water-limited environments. Adv Agron 71:193–231

Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphyt 135:255–263

Vinu V, Singh N, Vasudev S, Yadava DK, Kumar S, Naresh S, Bhat SR, Prabhu KV(2013) Assessment of genetic diversity in *Brassica juncea* (Brassicaceae) genotypes using phenotypic differences and SSR markers. Rev Biol Trop 61(4):1919–1934

Warwick SI (1993) Wild species in the tribe Brassicaceae (Cruciferae) as sources of agronomic traits. In: Guide to the wild germplasm of *Brassica* and allied crops. Technical Bulletin 17E, 119. Center for Land and Biological Resources Research Branch, Agriculture Canada, Ottawa, Ontario, Canada

Welcker C, Boussuge B, Benciveni C, RibautJM TF (2007) Are source and sink strengths genetically linked in maize plants subjected to water deficit? A QTL study of the responses of leaf growth and anthesis-silking interval to water deficit. J Exp Bot 58:339–349

Wright GC, Hubick KT, Farquhar GD (1988) Discrimination between carbon isotopes in leaves correlates with water use efficiency of field-grown peanut cultivars. Aust J Plant Physiol 15:815–825

Xiao QS, Zhang XK, Xu BB, Cheng Y, Zheng PY, Lu GY (2012) Cloning and expression pattern of AnnBn1gene in *Brassica napus*. Chin J Oil Crops Sci 34:123–128

Yamaguchi M, Sharp RE (2010) Complexity and coordination of root growth at low water potentials: recent advances from transcriptomic and proteomic analyses. Plant Cell Environ 33:590–603

Yang CJ, Zhang XK, Zou CS, Cheng Y, Zheng PY, Li GY (2007) Effects of drought simulated by PEG-6000 on germination and seedling growth of rapeseed (*Brassica napus*L.). Chin J Oil Crops Sci 29:425–430

Yang M, Yang Q, Fu T, Zhou Y (2011) Over expression of the Brassica napus BnLAS genein Arabidopsis affects plant development and increases drought tolerance. Plant Cell Rep 30:373–388

Yangj LD, Wang X, Ji C, Cheng F, Liu B, Hu Z, Chen S, Pental D, Ju Y, Yao P, Li X, Xie K, Zhang J, Wang J, Liu F, Ma W, Shopan J, Zheng N, Mackenzie SA, Zhang M (2016) The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. Nat Genet 48:1225–1232. https://doi.org/10.1038/ng.3657

Zaefizadeh M, Jamaati-e-Somarin S, Zabihie-Mahmoodabad R, Khayatnezhad M (2011) Discriminate analysis of the osmotic stress tolerance of different sub-cultivars of durum wheat during germination. Adv Environ Biol 5(1):74–80

Zakirullah Z, Swati ZA, Anwar A, Raziuddin Z (2000) Morpho-physiological response of selected brassica line to moisture stress. Pak J Biol Sci 3:130–132

Zhu ZH, Zheng WY, Zhang XK (2011) Principal component analysis and comprehensive evaluation on morphological and agronomic traits of drought tolerance in rapeseed (*Brassicanapus* L.). Sci Agri Sin 44:1775–1787

Chapter 6
Genomic Designing for Sesame Resistance to Abiotic Stresses

Xiurong Zhang, Jun You, Hongmei Miao, and Haiyang Zhang

Abstract Abiotic stress conditions result from climate change and the water-supply shortage affect plant growth and cause extensive losses to agricultural production worldwide. Sesame, one of the oldest and important oil-yielding crops, is highly valued for its high quality oil rich in antioxidants with health benefits. We describe here the abiotic stresses that significantly curtail the productivity of sesame and the progresses in the genetics and breeding research for abiotic stress tolerance improvement in sesame. The potential of genomics-assisted breeding for improvement ib abiotic stress tolerance in sesame is also discussed.

Keywords Sesame · *Sesamum indicum* · Abiotic stresses · Waterlogging · Drought · Breeding · QTLs · Genomics-assisted selection

6.1 Introduction

Sesame (*Sesamumindicum* L.), belonging to the genus *Sesamum*, is one of the world's most ancient oilseed crops with evidence that it has been cultivated in Asia for more than 5000 years (Bedigian 2004). Sesame is an annual self-pollinating oilseed crop and widely grown in tropical and subtropical areas mainly for its seed. Sesame seeds have higher oil content than other oilseed crops, contain approximately 55% oil and 25% protein (Wang et al. 2014). Besides high oil content, sesame is known for its nutritional and medicinal properties. The seed contains all essential amino acids and is a good source of unsaturated fatty acids and minerals such as calcium. It is worth noting that sesame seeds are also rich in antioxidants and bioactive compounds (such

---

X. Zhang (✉) · J. You
Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China
e-mail: zhangxr@oilcrops.cn

J. You
e-mail: junyou@caas.cn

H. Miao · H. Zhang
Henan Sesame Research Center, Henan Academy of Agricultural Sciences, Zhengzhou, China
e-mail: zhanghaiyang@zzu.edu.cn

C. Kole (ed.), *Genomic Designing for Abiotic Stress Resistant Oilseed Crops*,
https://doi.org/10.1007/978-3-030-90044-1_6

as sesamum, sesamolin, tocopherol and phytosterols) that are beneficial to human health (Pathak et al. 2014). Seed of sesame is widely used for edible products like edible oil, paste, cakes, flour, and confectioneries due to its high nutrients, unique taste and flavor (Hama 2016). Sesame oil also can be used for pharmaceutical or industrial uses such as raw material of cosmetics, soap, and lubricants (Myint et al. 2020).

Global sesame production was about 6.55 million tons in 2019, of which about 61% was produced in Africa and 34% in Asia (FAO 2019). Sudan is the world's largest producer of sesame, followed by Myanmar, India, Tanzania, Nigeria, China, Burkina Faso, Ethiopia, South Sudan, and Chad. With the improvement of consumers' health awareness and the deepening understanding of the benefits of sesame, the global demand for sesame is growing steadily (Dossa et al. 2017a; Myint et al. 2020). However, the abiotic stresses such as waterlogging and drought caused by climate anomalies seriously affect the yield and quality of sesame around the world.

6.2 Reduction in Yield and Quality Due to Abiotic Stresses in Sesame

6.2.1 Types and Distribution of Abiotic Stresses in Sesame

Sesame can grow in harsh environments and do not need much fertilizer or water. However, yield varies greatly with growing environment and cultivation practices. Waterlogging and drought are the main abiotic stresses in sesame. In China, the production of sesame often suffers from abiotic stresses during the growing season from June to August. About 20–35% and 10–30% of the planting areas suffer from waterlogging and drought, respectively. Waterlogging stress mainly occurred in Henan, Hubei, and Anhui provinces, occasionally a seasonal drought, while Liaoning, Hebei, Shanxi and Jiangxi were dominated by drought. Both waterlogging and drought stress occurred in the whole growth stage of sesame, and the frequency of waterlogging and drought stress occurred in different growth stage was 46% in seedling stage, 44% in early flowering stage, 52% in full flowering stage, 45% in final flowering stage, 41% in filling stage and 29% in maturity stage. In Myanmar, 18% of dryland sesame growers reported that excessive rainfall was the main cause of reduced dryland sesame production (Myint et al. 2020). Likewise, a short monsoon season resulting in drought stress also reduces the sesame yield in Myanmar (Myint and Kyaw 2019). In Ethiopia, sesame production is carried out under rain-fed conditions. Reduced rainfall and prolonged drought caused by climate change are the major challenges for sesame production in Ethiopia (Girmay 2018).

6.2.2 Waterlogging and Evaluation of Tolerance in Sesame

Sesame usually grows in rainfed regions. Waterlogging stress is the most common disaster for sesame (Fig. 6.1). Waterlogging stress leads to damage due to lack ofoxygen inplant tissues. For sesame, waterlogging inhibits the respiration of root, reduces the photosynthesis rate, inhibits the growth and development of plants, and finally cause serious yield losses (Wang et al. 2000; Sun et al. 2008, 2010b; Wei et al. 2013). Continuous waterlogging would reduce the yield and seed quality (Wang et al. 1999; Sun et al. 2010b; Sarkar et al. 2016; Yuan et al. 2018). Exposed with waterlogging, the yield of sesame could decrease by 44.8%–100% (Ding et al. 2012). Yuan et al. (2018) found that the plant height of six genotypes was reduced by 19.50%–46.76%, and the zone length of capsule was decreased by 24.02%–67.03% under 24–60 h waterlogging exposure. After waterlogging stress for 60 h, the plant yield of some varieties was reduced by 88.2%. On the other hand, the content of oil, protein, and polysaccharide in the six varieties varied from 51.99%–58.61%, 19.08%–22.05%, to 9.12%–13.68%, respectively. The content of fiber, polysaccharide, and ash also changed significantly in most varieties. For most test varieties treated under the 36 h waterlogging stress, the acid value and peroxide value of sesame oil varied significantly.

Morphological observations showed that the waterlogging tolerance was significantly correlated with root vigor, pubescence intensity on stem, and seed coat color (Liu et al. 1993; Wang et al. 2000). In order to evaluate waterlogging tolerance in

Fig. 6.1 Waterlogging stress in sesame field caused by excessive rainfall

sesame, Sun et al. (2010b) determined the capsule number per plant and seed weight per plant as the top two indicators for waterlogging tolerance assay from the 13 morphological and agronomic traits. Zhang et al. (2014) applied normal plant rate and plant survival rate to evaluate the waterlogging tolerance of sesameat flowering stage.

6.2.3 Drought and Evaluation for Tolerance in Sesame

Sesame originated from the tropical regions and has a certain drought tolerance. However, drought or water deficit also inhibits the growth and development of sesame plants (Fig. 6.2). Drought in the seedling stage leads to restrained growth and development of root and leaves, reduced plant height and biomass (Sun et al. 2010a; Harfi et al. 2016). Drought stress at flowering stage had significant effects on plant height, capsule size, seed per capsule and seed per plant, which resulted in decreased yield of sesame (Sun et al. 2010a; Golestani and Pakniyat 2015). Drought generally results in a reduction of 150–375 kg/ha, with an average reduction rate of about 23%. Serious drought can result in a 50–80% reduction of production. Eskandari et al. (2009) found that severe water stress reduced the yield of oil and protein by 38.18 and 10.77%, respectively, which affected the quality of sesame.

Fig. 6.2 Insufficient rainfall and lack of irrigation induced drought stress in sesame

Morphological comparison using scanning microscope reflected the spefic surface structure of leaf hairs and the variation among the various sesame varieties (Su et al. 2016). The variation of the structure and secretory components of sesame glandular hairs can be used to evaluate the drought resistance of sesame. Meanwhile, the amount of wax on leaves also indicate the tolerance level to drought stress and the seed yield (coefficient r = 0.466*) (Kim et al. 2007). In order to determine the ideal indicators for drought resistance in sesame, the effects of drought on growth and yield, biochemical and physio-morphological indices were evaluated (Sun et al. 2010a; Dossa et al. 2017c; Gholinezhad and Darvishzadeh 2018; Li et al. 2018a).

6.2.4 Strategies to Tackle Abiotic Stresses in Sesame

The counter measures to mitigate abiotic stresses include: (1) Planting varieties with strong stress resistance, which can increase the yield by 10–15% under stress condition; (2) use cultivation measures to deal with abiotic stress. To prevent and control waterlogging, the fields are usually made into "deep furrows and narrow block" or ridging, and clear the furrows in time for drainage after the rain. Measures such as mulching, drip irrigation and timely irrigation are often adopted to prevent and control drought. The application of these cultivation measures can increase production by 10–35%; and (3) spraying waterlogging-resistant inducers or drought-resistant agents on sesame leaves during the growth period, increasing production by 6–8%.

6.3 Traditional Breeding and Sesame Varieties with High Tolerance to Abiotic Stresses

6.3.1 Use of Morphological Markers

Identification of waterlogging tolerance of sesame was mainly carried out during germination and flowering stage. The evaluation index of waterlogging tolerance in germination period was relatively normal at seedling rate. The varieties with relatively normal seedling rate ≥80.00% was considered as high tolerance, 60.00–79.99% was tolerance, 40.00–59.99% was moderate tolerance, 20.00–39.99% was intolerance, and <20.00% was extremely intolerance. The evaluation index of waterlogging tolerance at the full flowering stage was relatively waterlogging-tolerance index, which was calculated by the number of withering plants, the withering grades and the number of surviving plants. The varieties with relative waterlogging-tolerance index ≥0.8 was considered as high tolerance, 0.6–0.79 was tolerance, 0.4–0.59 was moderate tolerance, 0.2–0.39 was intolerance, and <0.2 was extremely intolerance.

Drought tolerance of sesame was evaluated in germination stage and adult period. The evaluation index of drought tolerance at germination stage was relatively drought tolerance index, which was calculated by different drought tolerance grades and seedling numbers. The varieties with relatively drought tolerance index ≥90.00% was considered as high tolerance, 80.00–89.99% was tolerance, 70.00–79.99% was medium tolerance, 50.00–69.99% was intolerance, and <50.00% was extreme intolerance. The evaluation index of drought resistance at adult period was drought tolerance index, which was calculated by the number of withering plants, the withering grades and the number of surviving plants. The varieties with drought tolerance index ≥90.00% was considered as high tolerance, 70.00–89.99%, was tolerance, 50.00–69.99% was medium tolerance, 20.00–49.99% was intolerance, and <20.00% was extreme intolerance.

6.3.2 Breeding Objectives: Positive and Negative Selection

The positive selection targets in stress tolerance breeding include the survival rate and yield-related traits of plants under abiotic stress, which directly or indirectly reflects the resistance of plants to abiotic stress. The negative selection targets are traits related to quality and disease resistance, as well as yield under normal conditions. In other words, the improvement of stress tolerance should not bring negative effects on yield potential, quality and disease resistance.

6.3.3 Classical Breeding Achievements in Yield, Quality, and Stress Tolerance

The genetic improvement of tolerance of sesame in China began in the 1960s. At first, it mainly used line breeding methods. In the past decades, since the 1970s, a variety of breeding methods including conventional hybridization, radiation mutagenesis, space mutagenesis, line selection, distant hybridization, and the utilization of two-line heterosis of nuclear male sterility have been applied for sesame breeding. Some excellent Chinese elite varieties and local varieties, such as Zhongzhi 13, Yuzhi 1, Yiyangbai and Henan 1, showed high weather resistance and met the production requirements (Liu et al. 1993; Ding et al. 2012). Under the artificial waterlogging conditions, more representative varieties with high waterlogging tolerance such as Zhongzhi 5, Zhongzhi 7, Zhongzhi 11, Zhongzhi 13, Zhongzhi 20, Henan 1, Yiyangbai, Yuzhi 4, Zhengzhi 98N09, Zhengzhi 97C01, Zhengzhi 13, Ezhi 1, Ezhi 6, Jizhi 1, Luozhi 12, Zhuzhi 14, Zhuzhi 18 have been bred or screened. The drought-resistant varieties are Jinhuangma, Jinzhi 2, and Liaozhi 1 (data not shown, Xiongrong Zhang). Some sesame cultivars with tolerant to drought stress have been released in different states of India, such as Usha (OMT–11–6–5), Gouri, Madhavi, Uma (OMT–11–6–3), and Prachi (ORM 17) (Tripathy et al. 2019). Gholamhoseini

(2020) identified Sudan 94 as a drought-tolerant genotype with the best yield stability based on its agronomic traits under water deficiency.

6.3.4 Limitations of Traditional Breeding and Rationale for Molecular Breeding

Traditional breeding is a method of breeding new varieties based on phenotypic selection, which generally refers to selection, mutation and hybridization methods of breeding. One of the limitations of conventional breeding is that it is not easy to obtain the desired material with excellent characters, and the other is that the breeding cycle is long. Molecular breeding is based on molecular selection technology for the selection of new varieties, generally refers to molecular marker-assisted breeding. By using the genotypes of molecular markers closely linked to the target genes (or traits), molecular marker-assisted breeding can obtain individuals with the target genes (or traits) in the conventional breeding program through molecular selection, thus improving the selection efficiency and accelerating the breeding process. Transgenic breeding uses DNA recombination technology and DNA transfer technology to introduce the target gene into the recipient organism and obtain transgenic individuals, so as to realize directional breeding.

6.4 Genetic Diversity Related to Abiotic Stress Tolerance in Sesame

6.4.1 Phenotype-Based Diversity Analysis

The *Sesamum* genushas 23 species (IPGRI and NBPGR 2004)and *S. indicum* is the well-known and widely cultivated species within this genus. Several wild relatives of sesame with the adaptive features including hairiness, linear leaves, fleshy roots, more stomata located on the paraxial plane of leaf, and increased seed setting rate in dry season, have been proved tolerant to some abiotic stresses (Nimmakayala et al. 2011). For example, *S. laciniatum*, *S. occidentale* and *S. radiatum* were tolerant to drought stress, and *S. malabaricum* was reported resistant to waterlogging stress (Nimmakayala et al. 2011). These wild relatives of sesame are precious resources for abiotic stress tolerance improvement in sesame.

Beside wild related sesame species, over 25,000 genetic materials of cultivated sesame are currently preserved in some genebanks worldwide, including Oil Crops Research Institute, Chinese Academy of Agricultural Sciences in China, National Agrobiodiversity Center, Rural Development Administration in South Korea, and NBPGR National Gene Bank in India (Dossa et al. 2017a). Based on germplasm resources, several studies were performed to analyze the diversity of abiotic stress tolerance and screen for tolerance sources in sesame (Boureima et al. 2012, 2016; Zhang et al. 2014; Liu et al. 2017; Priyadharshini et al. 2018). Ding et al. (2012) evaluated the waterlogging tolerance of 43 main sesame cultivars from China at full flowering stage. They found most of the present sesame cultivars were sensitive to waterlogging stress, and two cultivars, Xiongzhi No. 1 and Zhongzhi No. 13, with higher percentage of normal plant and with higher harvest yield after waterlogging stress, respectively, showed relatively higher waterlogging tolerance among these cultivars. The results also indicated that waterlogging tolerance of the southern cultivars was higher than those from northern regions. Zhang et al. (2014) screened for tolerance sources of waterlogging stress in sesame core collections containing 186 landraces, and identified eight waterlogging tolerant germplasm. Liu et al. (2017) selected 12 sesame germplasms with high drought-tolerance from 100 sesame germplasm by a comprehensive evaluation method. Dossa et al. (2019a) analyzed the drought tolerance of 400 different sesame genotypes from 29 different countries around the world. Five traits associated with drought tolerance, including survival rate, stem length, capsule number, wilting level, and seed yield were investigated, and extensive variations of these traits were observed among the sesame genotypes under normal and drought stress condition. It was found that the drought resistance of the genotypes from tropical regions was significantly higher than that from northern regions. Li et al. (2018b) investigated the tolerance to drought and salinity of 490 sesame lines at germination stage. Most of the genotypes were moderately tolerant to drought and salt stresses, while the tolerant genotypes and sensitive ones were less represented for both stresses. In total, only 27 accessions were commonly tolerant to drought and salt stresses. Similarly, the correlation of traits between drought and salt was significantly weak, indicating the responses of different sesame genotypes to drought and excess salt stresses were quite distinctat germination stage.

6.4.2 Gene Pool of the Sesame Resources with High Toleranceto Abiotic Stresses

With continuous application of “omics” tools in sesame, more and more abiotic stress resistance-related gene resources have been discovered. Using whole-genome RNA-Seq analysis, Wang et al.(2012) identified 13,307 waterlogging-responsive genes in sesame. Later, a comparative time-course transcriptome analysis between waterlogging-sensitive and waterlogging-tolerant genotypes were performed to explore the molecular mechanisms of waterlogging stress response in sesame (Wang et al. 2016a). A total of 1379 genes, which were significantly differentially expressed at all time-point during waterlogging stress, were identified as the core genes responsible for the waterlogging response in sesame. Furthermore, 66 genes were identified as key components for improving waterlogging tolerance of sesame through a comparative analysis between two distinct genotypes. Recently, a high resolution dynamic transcriptome data of two contrasting sesame genotypes during the waterlogging and recovery stages were released (Dossa et al. 2019c). Clustering analysis of 126 RNA-seq data revealed three stages of sesame seed response to waterlogging stress: early response stage (0–12 h), delayed response stage (12–36 h) and recovery stage (36–48 h) (Wang et al. 2021a). Further analysis showed that WRKY and ERF transcription factor family members played an important role in transcriptional regulation of waterlogging-responsive genes during stress. By constructing a time-series expression regulation network of transcription factors and thier target genes, several key transcription factors, such as SiRAP2.2 and SiERF056, which simultaneously regulate the three waterlogging response periods, were discovered (Wang et al. 2021a). For drought stress, a RNA-seq analysis of sesame root identified 722 genes as core drought-responsesive genes and 61 genes showed different expression profiles in two sesame cultivars during drought stress (Dossa et al. 2017b). In another study, transcriptional profiling in sesame leaves of two contrasting genotypes for drought stress tolerance was characterized, and 684 up-regulated genes as well as 1346 down-regulated genes in both genotypes were revealed (You et al. 2019). Zhang et al. (2019a) analyzed the transcriptomic changes in sesame seedlings under salt stress. A total of 1946 and 1275 genes were identified in all time-point of salt-sensitive and salt-tolerant genotype, respectively. Notably, 59 genes were specific and robustly upregulated in salt tolerance genotypes under salt treatment, and were identified as resources for enhancing salt tolerance. Transcription factors play an important role in plant adaptation to abiotic stress. A series of TFs, such as ERF, bZIP, WRKY, MYB, NAC and HD-Zip, have been genome-wide analyzed in sesame, and several stress-responsive TF members of these family have been identified (Dossa et al. 2016; Li et al. 2017; Mmadi et al. 2017; Wang et al. 2018; Zhang et al. 2018; Wei et al. 2019). By a meta-analysis of sesame transcriptome datasets under drought, salt, waterlogging, and osmotic stresses, Dossa et al. (2019b) identified 543 genes as core abiotic stress-responsive genes (CARG) that robustly differentially expressed in all stress conditions. Transcription factor members belong to ERF, bHLH, MYB, and WRKY families were overrepresented in CARGs, indicating that these TF families are the main regulatory factors in response to various abiotic stresses in sesame. Moreover, overexpression of two transcription factors (SiERF5 and SiNAC104) in *Arabidopsis thaliana* increased tolerance to waterlogging, drought, and osmotic stresses. In another study, a R2-R3 MYB transcription factor, *SiMYB75*, strongly induced by drought, ABA, salinityand osmotic stresses was identified in sesame. Overexpression of *SiMYB75*in *Arabidopsis* increased ABA content and ABA sensitivity, as well as improved tolerance to salinity and drought stresses, suggesting that SiMYB75 modulates abiotic stresses through an ABA-dependent manner (Dossa et al. 2020). Chowdhury et al. (2017) overexpressed an osmotin-like gene from *Solanum nigrum* (SindOLP) in sesame. The transgenic sesame enhanced tolerance to salinity and drought stresses, as well as the charcoal rot pathogen through the integrated activation of multiple components of the defense signaling cascade.

6.5 Molecular Genetics and Breeding for Abiotic Stress Tolerance in Sesame

6.5.1 Mapping QTLs Related to Stress Tolerance in Sesame

Quantitative trait locus (QTL) mapping based on highdensity linkage maps is an approach widely used for investigating genetic variants responsible for phenotypic variation of complex traits. Zhang et al. (2014) mapped quantitative trait loci (QTLs) linked to waterlogging tolerance in sesame using a population of recombinant inbred lines derived from a cross between Zhongzhi 13 and Yiyangbai. The length of constructed genetic map was 592.4 cM, and 70 marker loci were distributed into 15 linkage groups (LGs), with an average distance of 8.46 cM. A total of six QTLs (*qWH09CHL15*, *qEZ09ZCL13*, *qEZ10CHL07*, *qEZ10ZCL07*, *qWH10CHL*09 and *qWH10ZCL09*) related to waterlogging tolerance at flowering stage were identified in sesame (Fig. 6.3), with individual QTLs explaining 5.67–17.19% of the phenotype variance. Furthermore, ZM428, a simple sequence repeat (SSR) marker tightly linked with *qWH10CHL09* (QTL explaining the most phenotype variance) was confirmed as an effective molecula rmarker for marker-assisted selection (MAS) to improve waterlogging tolerance of sesame.

6.5.2 Association of Molecular Markers and Target Genes Regulating Stress Tolerance in Sesame

Genome-wide association study (GWAS) has certain advantages over traditional linkage analysis and has been considered as apowerful tool for detecting the genetic architecture of complex traits in crops. Li et al. (2018b) performed GWAS in 490 diverse sesame accessions to analyze the genetic bases of drought (polyethylene glycol-induced) and salinity (NaCl-induced) tolerances at germination stage. There are 120 and 132 significant single nucleotide polymorphisms (SNPs) resolved to 15 and 9 QTLs identified for salinity and drought stresses, respectively. Only two QTLs were detected under both salinity and drought stress conditions, suggesting distinct genetic bases of salinity and drought tolerance in sesame. A total of 13 potential drought-tolerant genes and 27 potential salt-tolerant genes were identified in the QTL region, closely involving in signal transduction, hormone synthesis or ion sequestration. Dossa et al. (2019a) investigated the genetic basis of sesame drought tolerance at flowering stage by GWAS based on drought tolerance related traits (survival rate, stem length, capsule number, wilting level, and yield in control and stress conditions). Ten stable QTLs (constitutively detected in two years or different traits) located in four LGs explained more than 40% of phenotypic variation. Two pleiotropic QTLs harboring known and unreported genes related to drought resistance, including *SiSAM*, *SiABI4*, *SiGOLS1*, *SiTTM3*, and *SiNIMIN1* were reported.

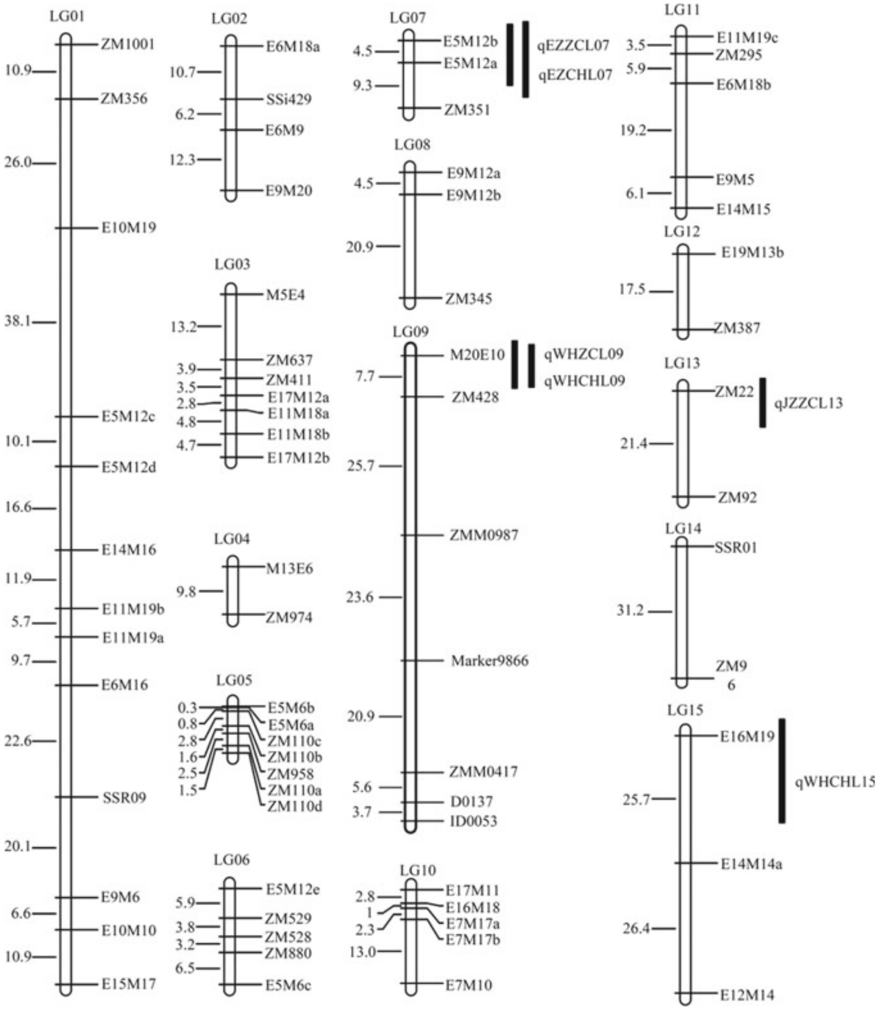

Fig. 6.3 Mapping of sesame waterlogging tolerance QTL (Zhang et al. 2014)

Moreover, the authors found that a missense mutation in the coding region of *SiSAM* may contribute to the natural variation of sesame drought tolerance.

6.6 Genomics-Aided Breeding for Stress ToleranceTraits

Research on genetics and molecular biology of sesame was almost blank before 2010. However, the release of the draft sesame genome (Wang et al. 2014) and the application of various omics technologies (Wei et al. 2011, 2014b, 2015; Zhang et al.

2019b) have greatly promoted the research on genetics and functional genomics in sesame. With these invaluable efforts, a large number of genetic resources included informative molecular markers (Zhang et al. 2012; Wei et al. 2014a, b; Dossa 2016; Purru et al. 2018; Kizil et al. 2020), ultra-dense genetic maps (Wang et al. 2016b; Zhang et al. 2016; Mei et al. 2017), transcriptome assemblies (Wei et al. 2011; Wang et al. 2012; Dossa et al. 2017b, 2019c; You et al. 2019; Zhang et al. 2020), integrative online databases (Wang et al. 2015, 2021b; Dossa et al. 2017d; Wei et al. 2017) etc. were developed in sesame, which provide an important basis for the genetic improvement of important agronomic traits including abiotic stress resistance in sesame.

Marker-assisted selection (MAS) is an indirect selection process based on molecular markers associated with the traits of interest, which makes efficient selection in breeding programs. Although numerous QTLs for traits associated with abiotic stress tolerance were identified invarious crops, few of them were successfully used in stress tolerance breeding mainly due to strong genotype-by-environment interaction (Mishra et al. 2013; Priyadarshan 2019). Some SSR or SNP markers associated with abiotic stress tolerance related traits were detected in sesame (Zhang et al. 2014; Li et al. 2018b; Dossa et al. 2019a), but their effectiveness in breeding for stress tolerance through MAS needs further evaluation. Besides MAS, transgenic techniqueisalsoan efficient way to enhance resistance to abiotic stresses in crop breeding. Although the protocol of genetic transformation through *Agrobacterium* in sesame need to be further optimized, some successful attempts have provided opportunities for improve abiotic stress tolerance of sesame by transgenic breeding (Yadav et al. 2010; Al-Shafeay et al. 2011; Chowdhury et al. 2014). In a recent study, an exogenous gene from *Solanum nigrum* was introduced into sesame that enhanced stress and disease resistance in transgenic plants (Chowdhury et al. 2017).

Global planting area and production of sesame have remarkably increased in recent years, but the productivity of sesame is still very low mainly due to its poor yield stability in various adverse conditions. Over the last two decades, substantial progress has been made in revealing the genetic basis of traits related to abiotic stress tolerance and molecular mechanisms of tolerance to abiotic stress in sesame. More importantly, several QTLs and functional genes related to abiotic stress tolerance were identified and could be used in breeding progress. More genetic sources of tolerance to abiotic stresses characterized in valuable germplasm resources, and more applications of molecular breeding technique, will help to accelerate the genetic improvement of abiotic stress tolerance in sesame.

References

Al-Shafeay AF, Ibrahim AS, Nesiem MR, Tawfik MS (2011) Establishment of regeneration and transformation system in Egyptian sesame (*Sesamum indicum* L.) cv Sohag 1. GM Crops 2(3):182–192

Bedigian D (2004) History and lore of sesame in Southwest Asia. Econ Bot 58(3):329–353

Boureima S, Oukarroum A, Diouf M, Cisse N, Van Damme P (2012) Screening for drought tolerance in mutant germplasm of sesame (*Sesamum indicum*) probing by chlorophyll a fluorescence. Environ Exp Bot 81:37–43

Boureima S, Diouf M, Amoukou A, Van Damme P (2016) Screening for sources of tolerance to drought in sesame induced mutants: assessment of indirect selection criteria for seed yield. Intl J Pure Appl Biosci 4(1):45–60

Chowdhury S, Basu A, Kundu S (2017) Overexpression of a new osmotin-like protein gene (SindOLP) confers tolerance against biotic and abiotic stresses in sesame. Front Plant Sci 8:410

Chowdhury S, Basu A, Kundu S (2014) A new high-frequency *Agrobacterium*-mediated transformation technique for *Sesamum indicum* L. using de-embryonated cotyledon as explant. Protoplasma 251(5):1175–1190

Ding X, Wang L, Zhang Y, Li D, Wei W, Zhang X (2012) Evaluation of the waterlogging tolerance of the main sesame cultivars in China. Acta Agri Bor-Sin 27(4):89–93. (in Chinese with English abstract)

Dossa K (2016) A physical map of important QTLs, functional markers and genes available for sesame breeding programs. Physiol Mol Biol Plants 22(4):613–619

Dossa K, Wei X, Li D, Fonceka D, Zhang Y, Wang L, Yu J, Boshou L, Diouf D, Cisse N, Zhang X (2016) Insight into the AP2/ERF transcription factor superfamily in sesame and expression profiling of DREB subfamily under drought stress. BMC Plant Biol 16(1):171

Dossa K, Diouf D, Wang L, Wei X, Zhang Y, Niang M, Fonceka D, Yu J, Mmadi MA, Yehouessi LW, Liao B, Zhang X, Cisse N (2017a) The emerging oilseed crop *Sesamum indicum* enters the "omics" era. Front Plant Sci 8:1154

Dossa K, Li D, Wang L, Zheng X, Liu A, Yu J, Wei X, Zhou R, Fonceka D, Diouf D, Liao B, Cisse N, Zhang X (2017b) Transcriptomic, biochemical and physio-anatomical investigations shed more light on responses to drought stress in two contrasting sesame genotypes. Sci Rep 7(1):8755

Dossa K, Yehouessi L, Likeng-Li-Ngue B, Diouf D, Liao B, Zhang X, Cissé N, Bell J (2017c) Comprehensive screening of some west and central african sesame genotypes for drought resistance probing by agromorphological, physiological, biochemical and seed quality traits. Agronomy 7(4):83

Dossa K, Yu J, Liao B, Cisse N, Zhang X (2017d) Development of highly informative genome-wide single sequence repeat markers for breeding applications in sesame and construction of a web resource: SisatBase. Front Plant Sci 8:1470

Dossa K, Li D, Zhou R, Yu J, Wang L, Zhang Y, You J, Liu A, Mmadi MA, Fonceka D, Diouf D, Cisse N, Wei X, Zhang X (2019a) The genetic basis of drought tolerance in the high oil crop *Sesamum indicum.* Plant Biotechnol J 17(9):1788-1803

Dossa K, Mmadi MA, Zhou R, Zhang T, Su R, Zhang Y, Wang L, You J, Zhang X (2019b) Depicting the core transcriptome modulating multiple abiotic stresses responses in sesame (*Sesamum indicum* L.). Intl J Mol Sci 20(16):3930

Dossa K, You J, Wang L, Zhang Y, Li D, Zhou R, Yu J, Wei X, Zhu X, Jiang S, Gao Y, Mmadi MA, Zhang X (2019c) Transcriptomic profiling of sesame during waterlogging and recovery. Sci Data 6(1):204

Dossa K, Mmadi MA, Zhou R, Liu A, Yang Y, Diouf D, You J, Zhang X (2020) Ectopic expression of the sesame MYB transcription factor SiMYB305 promotes root growth and modulates ABA-mediated tolerance to drought and salt stresses in Arabidopsis. AoB Plants 12(1):plz081

Eskandari H, Zehtabsalmasi S, Ghassemigolezani K, Gharineh MH (2009) Effects of water limitation on grain and oil yields of sesame cultivars. J Food Agric Environ 7(2):339–342

FAO (2019) Food and agriculture organization statistical databases (FAOSTAT). FAOSTAT provides free access to food and agriculture data for over 245 countries and territories and covers all FAO regional groupings. Available at: http://faostat.fao.org/

Gholamhoseini M (2020) Evaluation of sesame genotypes for agronomic traits and stress indices grown under different irrigation treatments. Agron J 112(3):1794–1804

Gholinezhad E, Darvishzadeh R (2018) Investigation the drought tollerance of sesame (*Sesamium indicum* L.) local landraces based on drought stress tolerance indices in different levels of irrigation and mycorrhizae. J Crop Breeding 10(26):185–194. (in Persian with English abstract)

Girmay AB (2018) Sesame production, challenges and opportunities in Ethiopia. Vegetos 31(1):51

Golestani M, Pakniyat H (2015) Evaluation of traits related to drought stress in sesame (*Sesamum Indicum* L.) genotypes. J Asian Sci Res 5(9):465–472

Hama JR (2016) Comparison of fatty acid profile changes between unroasted and roasted brown sesame (*Sesamum indicum* L.) seeds oil. Int J Food Prop 20(5):957–967

Harfi ME, Hanine H, Rizki H, Latrache H, Nabloussi A (2016) Effect of drought and salt stresses on germination and early seedling growth of different color-seeds of sesame (*Sesamum indicum*). Intl J Agri Biol 18(06):1088–1094

IPGRI, NBPGR (2004) Descriptors for Sesame (*Sesamum* spp.). International Plant Genetic Resources Institute, Rome, Italy and National Bureau of Plant Genetic Resources, New Delhi, India

Kim KS, Park SH, Jenks MA (2007) Changes in leaf cuticular waxes of sesame (*Sesamum indicum* L.) plants exposed to water deficit. J Plant Physiol 164(9):1134–1143

Kizil S, Basak M, Guden B, Tosun HS, Uzun B, Yol E (2020) Genome-wide discovery of InDel markers in sesame (*Sesamum indicum* L.) using ddRADSeq. Plants 9(10):1262

Li D, Liu P, Yu J, Wang L, Dossa K, Zhang Y, Zhou R, Wei X, Zhang X (2017) Genome-wide analysis of WRKY gene family in the sesame genome and identification of the WRKY genes involved in responses to abiotic stresses. BMC Plant Biol 17(1):152

Li D, Dossa K, Zhang Y, Wang L, Zhu X, Wang L, Zhang X (2018a) Biochemical and physio-morphological indices for sesame drought resistance germplasm selection. Chin J Oil Crop Sci 3:99-110. (in Chinese with English abstract)

Li D, Dossa K, Zhang Y, Wei X, Wang L, Zhang Y, Liu A, Zhou R, Zhang X (2018b) GWAS uncovers differential genetic bases for drought and salt tolerances in sesame at the germination stage. Genes 9(2):87

Liu J, Tu L, Xu R, Zheng Y (1993) The relationship between the waterlogging resistance and the genotypes and the vigor of root system in sesame (*Sesamum indicum* L.). Acta Agri Bor-Sin 3:82-86. (in Chinese with English abstract)

Liu W, Lü W, Li D, Ren G, Zhang Y, Wen F, Han J, Zhang X (2017) Drought resistance of sesame germplasm resources and association analysis at adult stage. Sci Agri Sin 50(4):625-639. (in Chinese with English abstract)

Mei H, Liu Y, Du Z, Wu K, Cui C, Jiang X, Zhang H, Zheng Y (2017) High-density genetic map construction and gene mapping of basal branching habit and flowers per leaf axil in sesame. Front Plant Sci 8:636

Mishra KK, Vikram P, Yadaw RB, Swamy BP, Dixit S, Cruz MT, Maturan P, Marker S, Kumar A (2013) qDTY12.1: a locus with a consistent effect on grain yield under drought in rice. BMC Genet 14:12

Mmadi MA, Dossa K, Wang L, Zhou R, Wang Y, Cisse N, Sy MO, Zhang X (2017) Functional characterization of the versatile MYB gene family uncovered their important roles in plant development and responses to drought and waterlogging in sesame. Genes (basel) 8(12):362

Myint D, Gilani SA, Kawase M, Watanabe KN (2020) Sustainable sesame (*Sesamum indicum* L.) production through improved technology: an overview of production, challenges, and opportunities in Myanmar. Sustainability 12(9):3515

Myint T, Kyaw EMT (2019) Assessment of supply chain management of sesame seed in Pakokku township, Magway region, Myanmar. Int J Agri Market 6:215–224

Nimmakayala P, Perumal R, Mulpuri S, Reddy UK (2011) Sesamum. In: Kole C (ed) Wild corp relatives: genomic and breeding resources, vol Oilseeds. Springer, Berlin, pp 261–273

Pathak N, Rai AK, Kumari R, Bhat KV (2014) Value addition in sesame: A perspective on bioactive components for enhancing utility and profitability. Pharmacogn Rev 8(16):147–155

Priyadarshan PM (2019) Breeding for abiotic stress Adaptation. Plant breeding: classical to modern. Springer, Singapore, pp 413–455

Priyadharshini B, Prakash M, Vignesh M, Murugan S, Anandan R (2018) Multivariate analysis of sesame genotypes under saline stress. Indian J Agri Res 52:708–711

Purru S, Sahu S, Rai S, Rao AR, Bhat KV (2018) GinMicrosatDb: a genome-wide microsatellite markers database for sesame (*Sesamum indicum* L.). Physiol Mol Biol Plants 24(5):929–937

Sarkar PK, Khatun A, Singha A (2016) Effect of duration of waterlogging on crop stand and yield of sesame. Intl J Innov Appl Stud 14:1–6

Su S, Li R, Lang D, Zhang K, Hao X, Liu Y, Wang J, Zhang H, Xu H (2016) Microstructure of glandular trichomes on leaf surface of sesame and changes of trichome secretions under drought condition. Acta Agronl Sinl 42:278–294. (in Chinese with English abstract)

Sun J, Zhang X, Zhang Y, Huang B, Che Z (2008) Comprehensive evaluation of waterlogging tolerance of different sesame varieties. Chin J Oil Crop Sci 30:518–521+528. (in Chinese with English abstract)

Sun J, Rao Y, Le M, Yan T, Yan X, Zhou H (2010a) Effects of drought stress on sesame growth and yield characteristics and comprehensive evaluation of drought tolerance. Chinl J l Oil Crop Sci 32:525–533. (in Chinese with English abstract)

Sun J, Zhang X, Zhang Y, Wang L, Li D (2010b) Evaluation of yield characteristics and waterlogging tolerance of sesame germplasm with different plant types after waterlogging. J Plant Genet Resour 11:139–146. (in Chinese with English abstract)

Tripathy SK, Kar J, Sahu D (2019) Advances in sesame (*Sesamum indicum* L.) breeding. In: Al-Khayri J, Jain S, Johnson D (eds) Advances in plant breeding strategies: industrial and food crops. Springer, Cham, pp 577–635

Wang L, Yu S, Tong C, Zhao Y, Liu Y, Song C, Zhang Y, Zhang X, Wang Y, Hua W, Li D, Li D, Li F, Yu J, Xu C, Han X, Huang S, Tai S, Wang J, Xu X, Li Y, Liu S, Varshney RK, Wang J, Zhang X (2014) Genome sequencing of the high oil crop sesame provides insight into oil biosynthesis. Genome Biol 15(2):R39

Wang W, Mei H, Zheng Y, Zhang F (1999) Study on response to waterlogging and adaptative change in sesame (*Sesamum indicum* L.) I. Changes of morphology, biomass and seed yield of different sesame genotypes under artificial flooding condition. Chin J Oil Crop Sci 4. (in Chinese with English abstract)

Wang W, Zheng Y, Mei H, Zhang F (2000) Studies on response to waterlogging and adaptive change in sesame (*Sesamum indicum* L.) II. Effects of waterlogging and growth regulators on physiological characteristics of some sesame genotypes. Chin J Oil Crop Sci 2. (in Chinese with English abstract)

Wang L, Zhang Y, Qi X, Li D, Wei W, Zhang X (2012) Global gene expression responses to waterlogging in roots of sesame (*Sesamum indicum* L.). Acta Physiologiae Plantarum 34(6):2241–2249

Wang L, Yu J, Li D, Zhang X (2015) Sinbase: an integrated database to study genomics, genetics and comparative genomics in *Sesamum indicum*. Plant Cell Physiol 56(1):e2

Wang L, Li D, Zhang Y, Gao Y, Yu J, Wei X, Zhang X (2016a) Tolerant and susceptible sesame genotypes reveal waterlogging stress response patterns. PLoS One 11(3):e0149912

Wang L, Xia Q, Zhang Y, Zhu X, Zhu X, Li D, Ni X, Gao Y, Xiang H, Wei X, Yu J, Quan Z, Zhang X (2016b) Updated sesame genome assembly and fine mapping of plant height and seed coat color QTLs using a new high-density genetic map. BMC Genomics 17:31

Wang Y, Zhang Y, Zhou R, Dossa K, Yu J, Li D, Liu A, Mmadi MA, Zhang X, You J (2018) Identification and characterization of the bZIP transcription factor family and its expression in response to abiotic stresses in sesame. PLoS One 13(7):e0200850

Wang L, Dossa K, You J, Zhang Y, Li D, Zhou R, Yu J, Wei X, Zhu X, Jiang S, Gao Y, Mmadi MA, Zhang X (2021a) High-resolution temporal transcriptome sequencing unravels ERF and WRKY as the master players in the regulatory networks underlying sesame responses to waterlogging and recovery. Genomics 113(1):276–290

Wang L, Yu J, Zhang Y, You J, Zhang X, Wang L (2021b) Sinbase 2.0: an updated database to study multi-omics in *Sesamum indicum*. Plants 10(2):272

Wei W, Li D, Wang L, Ding X, Zhang Y, Gao Y, Zhang X (2013) Morpho-anatomical and physiological responses to waterlogging of sesame (*Sesamum indicum* L.). Plant Sci 208:102–111

Wei L, Miao H, Li C, Duan Y, Niu J, Zhang T, Zhao Q, Zhang H (2014) Development of SNP and InDel markers via de novo transcriptome assembly in *Sesamum indicum* L. Mol Breed 34(4):2205–2217

Wei X, Liu K, Zhang Y, Feng Q, Wang L, Zhao Y, Li D, Zhao Q, Zhu X, Zhu X, Li W, Fan D, Gao Y, Lu Y, Zhang X, Tang X, Zhou C, Zhu C, Liu L, Zhong R, Tian Q, Wen Z, Weng Q, Han B, Huang X, Zhang X (2015) Genetic discovery for oil production and quality in sesame. Nat Commun 6:8609

Wei X, Gong H, Yu J, Liu P, Wang L, Zhang Y, Zhang X (2017) SesameFG: an integrated database for the functional genomics of sesame. Sci Rep 7(1):2342

Wei M, Liu A, Zhang Y, Zhou Y, Li D, Dossa K, Zhou R, Zhang X, You J (2019) Genome-wide characterization and expression analysis of the HD-Zip gene family in response to drought and salinity stresses in sesame. BMC Genomics 20(1):748

Wei W, Qi X, Wang L, Zhang Y, Hua W, Li D, Lv H, Zhang X (2011) Characterization of the sesame (*Sesamum indicum* L.) global transcriptome using Illumina paired-end sequencing and development of EST-SSR markers. BMC Genomics 12:451

Wei X, Wang L, Zhang Y, Qi X, Wang X, Ding X, Zhang J, Zhang X (2014b) Development of simple sequence repeat (SSR) markers of sesame (*Sesamum indicum*) from a genome survey. Molecules 19(4):5150–5162

Yadav M, Chaudhary D, Sainger M, Jaiwal PK (2010) *Agrobacterium tumefaciens*-mediated genetic transformation of sesame (*Sesamum indicum* L.). Plant Cell Tiss Org Cult 103(3):377–386

You J, Zhang Y, Liu A, Li D, Wang X, Dossa K, Zhou R, Yu J, Zhang Y, Wang L, Zhang X (2019) Transcriptomic and metabolomic profiling of drought-tolerant and susceptible sesame genotypes in response to drought stress. BMC Plant Biol 19(1):267

Yuan Q, Zhang H, Miao H, Duan Y, Wei Q, Wang X (2018) Effects of waterlogging stress on the quality of sesame seed and oil product. Acta Agri Bor-Sin 33:202–208. (in Chinese with English abstract)

Zhang H, Wei L, Miao H, Zhang T, Wang C (2012) Development and validation of genic-SSR markers in sesame by RNA-seq. BMC Genomics 13:316

Zhang H, Miao H, Li C, Wei L, Duan Y, Ma Q, Kong J, Xu F, Chang S (2016) Ultra-dense SNP genetic map construction and identification of SiDt gene controlling the determinate growth habit in *Sesamum indicum* L. Sci Rep 6:31556

Zhang Y, Wang L, Li D, Gao Y, Lü H, Zhang X (2014) Mapping of sesame waterlogging tolerance QTL and identification of excellent waterlogging tolerant germplasm. Sci Agri Sin 47(3):422-430. (in Chinese with English abstract)

Zhang Y, Li D, Wang Y, Zhou R, Wang L, Zhang Y, Yu J, Gong H, You J, Zhang X (2018) Genome-wide identification and comprehensive analysis of the NAC transcription factor family in *Sesamum indicum.* PLoS One 13(6):e0199262

Zhang Y, Li D, Zhou R, Wang X, Dossa K, Wang L, Zhang Y, Yu J, Gong H, Zhang X, You J (2019a) Transcriptome and metabolome analyses of two contrasting sesame genotypes reveal the crucial biological pathways involved in rapid adaptive response to salt stress. BMC Plant Biol 19(1):66

Zhang Y, Wei M, Liu A, Zhou R, Li D, Dossa K, Wang L, Zhang Y, Gong H, Zhang X, You J (2019b) Comparative proteomic analysis of two sesame genotypes with contrasting salinity tolerance in response to salt stress. J Proteom 201:73–83

Zhang Y, Li D, Zhou R, Liu A, Wang L, Zhang Y, Gong H, Zhang X, You J (2020) A collection of transcriptomic and proteomic datasets from sesame in response to salt stress. Data in Brief 32:106096

Chapter 7
Abiotic Stresses in Castor Plant

Xuegui Yin, Jiannong Lu, Akwasi Yeboah, and Yuelian Liu

Abstract Castor, *Ricinus communis,* is one of the top ten oil crops in the world. It has been paid more and more attention because of its high economic value. In the process of growth and development, it is subjected to a variety of abiotic stresses from the environment. In this chapter, the stresses on castor are discussed in consideration of heat tolerances, cold tolerance, drought tolerance, flooding and submergence tolerance, nutrient use efficiency, water use efficiency, salt-alkali stress and metal ion toxicity. It is suggested that more attention should be paid to the physiological adaptation mechanisms of castor to these stresses.

Keywords *Ricinus communis* L. · Abiotic atress · Heat tolerance · Cold tolerance · Drought tolerance · Salinity tolerance · Metal tolerance

7.1 Introduction

Castor is good at resistance to stresses, and is good at adaptability to soil drought, salinity, alkalinity and other adverse conditions (Jiao et al. 2019). In the recent years, it has also been widely used in soil improvement and ecological environment construction (Zhou et al. 2010; Wang et al. 2019). Therefore, it is necessary to study the mechanism of abiotic stresses on castor. In base of this, this chapter systematically summarized the research progress of abiotic stresses on castor. Further, it was put forward the research prospect, aiming to provide a scientific reference for the theoretical research of castor abiotic stress and the application in production practice (Blum 2017).

X. Yin (✉) · J. Lu · A. Yeboah · Y. Liu
College of Agricultural Sciences, Guangdong Ocean University, Zhanjiang, Guangdong, China
e-mail: yinxuegui@126.com

C. Kole (ed.), *Genomic Designing for Abiotic Stress Resistant Oilseed Crops*,
https://doi.org/10.1007/978-3-030-90044-1_7

7.2 Root Characters

Castor plant has a well-developed tap root system, with primary root and all forms of lateral roots. The primary root can reach up to 4 m underground in soil from which about three to six primary lateral roots spread out. Out of the primary lateral roots, the secondary, tertiary, and quaternary lateral roots are produced. The secondary root is mostly found at the soil surface and travels parallel to soil surface, with a little bend downward, and grows to 90–120 cm. The tertiary roots grow to 30–45 cm long (Naik 2018). Well-developed lateral roots of the plant can grow up to 2 m in soil. The development of root hairs and fine root from the primary and lateral roots form coniform root system (Moshkin 1986). Majority of the plant roots dispense within 50 cm from the surface of the earth and penetrate deeper into the soil during low rainy seasons. The lateral roots can be visible during moist conditions. Castor root characters is one of its major tolerance to several abiotic stressors such as drought and heavy metals (Salihu et al. 2014; Yeboah et al. 2020).

7.3 Heat Tolerance

Plant growth and development are affected by high temperatures. During plant growth periods, the amount of heat unit needed by the plant depends on the climatic conditions (Hasanuzzaman et al. 2013). Castor bean is a thermophilic crop and its response to heat stress varies among geographical origins (Severino and Auld 2013a). In the tropical and subtropical regions, cultivars from the temperate region usually experience senescence or premature death. The vigorous growth season of castor is in June to August in China. Once the temperature exceeds 40°C, the flower buds and flowers will wilt, and the ovary will fall, which will affect the seed setting during the season (Liu et al. 2005). In Southern part of China, sowing of castor seeds in September–October extend the growth period with increase capsule formation and seed yield compared to spring sowing in February–March which is attributed to the lower temperature, and sowing in July–August leads to varieties with smaller racemes than normal due to the relatively high temperature (Yin et al. 2019). At seed filling stage, heat stress directly impedes yield, as increased senescence hinders seed setting, resulting in weight loss (Severino and Auld 2013a). Exposure to high temperature hinders several metabolic processes including transpiration, respiration, membrane thermo-stability, and photosynthesis by interfering the electron transfer mediated by PSII, transforming the fluidity of thylakoid membranes, which can lead to the ooze of PSII light-harvesting complexes and decrease chlorophyll (Hasanuzzaman et al. 2013; Wahid et al., 2007). Several mechanisms are involved to enhance plants growth upon heat stress. Acquired tolerance mechanism also known as avoidance/acclimation, is where plants survive under lethal temperatures after being exposed to mild temperature, and inherited tolerance also known as adaptation mechanism involves the survival of plants under extremely high temperature (Wahid et al. 2007). Traits including alteration of membrane lipid compositions, leaf cooling via transpirational loss, and change in leaf orientation are observed to select avoidance mechanism, and trait for adaptation mechanisms like early maturity that is related to smaller yield losses which is observed in most crops could be explored in castor (Lipiec et al. 2013; Wahid et al. 2007).

7.4 Cold Tolerance

Different plant species especially crop cultivars require a specific scope of temperatures for their normal functioning. These temperatures rely not only on the cultivar but also on the growth stage of a particular cultivar (Sala et al. 2012). A deviation from the optimum range of temperature results in temperature stress, that is, it disrupts plant performance. Stressors due to low temperature can be classified into two namely chilling stress and freezing stress (Kolaksazov et al. 2013).

Castor is very susceptible to cold stress at the early growth stage. For this reason, the slow germination, irregular germination and cold-sensitive germination of the seed often exist, which is one of the major concerns that has attracted the attention of many breeders but yet to no avail. Temperature around 14 to 15 °C enhances its germination, the optimum and maximum temperatures needed by the plant are 31 and 36 °C, respectively (Salihu et al. 2014). Within this scope, the germination rate and emergence rate increase as the temperature increases. Germination and seedling emergence delays when grown in soil with low temperature which leads to irregular stands (Moshkin 1986).

Since the plant is highly sensitive to cold, temperature below −1 °C, capable to induce frost, may cause severe damage or death to castor seedlings. The plant is affected by cold when temperature drops to 5ºC or below (Moshkin 1986). On an average, daily mean temperature of 15 °C enhances the seedling growth and optimum temperature required for the overall growth period fall between 20 and 28 °C. To a certain extent, an increase in temperature may reduce the flowering and maturity period. The pistil and stamen flowers bloom provided the daily mean temperature is greater than 18 °C (Yin et al. 2019).

In order to increase castor tolerance to cold stress, breeding of cold tolerant cultivars and some physiological parameter may be useful to select for resistant varieties. Parameters including the content of chlorophyll, specific leaf area etc. correlated genetically to cold stress (Škorić 2016) and these criteria can be studied in castor to enhance tolerance to cold stress.

7.5 Drought Tolerance

The ability of plants to grow and survive when faced with scarcity of water resources can be termed as drought tolerance. Drought is one of the major factors that caused great famine in the past and could still be a crucial threat to the world food security. Castor as an industrial crop is tolerant to drought owing to its strong root system which can penetrate deeper into the soil. The ability of castor to cope in drought area is beneficial which will help lessen the competition for land with other food crops.

At the early growth stages, castor plant is highly sensitive to water stress. The callus initiation, chlorophyll content, and nitrate reductase activity in castor reduced due to water stress, at the cellular level (Manjula et al. 2003). Drought stress increases the abscisic acid level in the phloem sap as well as the cuticular wax load (Zhong et al. 1996; Lakshmamma et al. 2009).

One mechanism castor plant adopts to tolerate drought is by osmotic adjustment which sustains water relations under osmotic stress. Osmotic adjustment accumulates a range of osmotically active molecules/ions such as soluble sugars, proline, organic acids, chloride ions, calcium, and potassium (Shanker et al. 2014). The osmotic potential of the cell becomes minimized under limited water supply in order to pull water into the cell and helps maintain turgor. By osmotic adjustment, the cytoplasmic activities and organelles occur at a normal pace and it increases plant growth, assimilation, and photosynthesis activities (Blum 2017). The presence of osmotic adjustments in the leaves of castor greatly varied with the severity of the effect genotypes. Accumulation of soluble sugars were the highest (61%) in the osmotically active compounds accumulated by the genotypes, and then the free amino acids (17%) and proline (12%), and the least with 2.8% for potassium (Babita et al. 2010). The genotypes with the highest seed yield of osmotic adjustment was 53% which was higher than those with low osmotic adjustment (Babita et al. 2010).

Under water deficit, the stomatal conductance was maintained and the net CO_2 fixation of castor plant was high without being affected. The early stomatal closure decreased the rate of water loss by transpiration (Sausen and Rosa 2010). Photosynthetic contents of castor plants observed under drought stress was protected and restrictions of photosynthetic pigment were usually due to diffusive resistance (Sausen and Rosa 2010). Severe drought stress partially reduced the photosynthetic functions of castor, however, after 24 h of exposure to this stress, the plant fully regained its normal photosynthetic function. But, the plant was highly sensitive to limited light (Funk and Zachary 2010).

7.6 Flooding and Submergence Tolerance

Despite water being a requirement for plant growth and development, its excess can negatively affect their viability and productivity (Fukao et al. 2019). The unpredicted occurrences, localizations, regimes, and global warming have increased floods

(Wilson et al. 2019). About 10% of the world's land area is waterlogged and this has become one of the most crucial restrictions affecting crop production (Patel et al. 2015). In determining plant survival to flood, factors such as type of crop and soil, growth stage, flooding conditions, and fertility levels are considered (Fukao et al. 2019).

Castor is a non-waterlogging crop and highly responsive to soil anoxia caused by waterlogging. After the exposure of castor plants to soil flooding for a minimum of 2 h and maximum for 6 h, the amount of abscisic acid produced by flooded roots, CO_2 uptake, leaf extension, hydraulic conductance of root, stomatal conductance, and transpiration were decreased (Else et al. 2001). Castor beans exposured to soil flooding after 3 days were completely injuerd and died after 4 days (Severino et al. 2005). Plants left exposed to hypoxic conditions revealed that the amount of starch, β-amylase activity, protein, and soluble sugars increased as the nitrate reductase activity and seed yield did reduced (Beltrão et al. 2006; Baldwin and Cossar 2009).

Although it has been concluded by experiments that flooding affects castor growth and development, the plant response to excessive water ramains unknown, the physiological mechanisms for responses of plant to excessive soil moisture need further studies.

7.7 Nutrient Use Efficiency

The nutrient use efficiency (NUE) of plants depends on the amount and availability of nutrient intake, integration, and utilization capacity by the plant (Aouass et al. 2020). Variation in the use of nutrient among plants might be as a result of the inherent genetic nature of the germplasm resources, which successively reflect on the effectiveness of nutrient uptake, absorption, production of dry matter, shoot and leaves translocation efficiency, among others and also affects the interactions between plant and its environment (Baligar et al. 2001; Baligar and Fageria 2015). Over the years, the appilication of mineral fertilizers such as nitrogen, phosphorus, and potassium have been beneficial to increase global food production. However, environmental stressors can inflict numerous challenges like nutrient uptake, cycling, and availability which particularly influences plant NUE, thereby decreasing plant growth, development and consequently the entire yield (Panjabi et al. 2019). The effect of atmospheric CO_2 and high-temperature stress owing to drought also can influence the seed yield and oil content of castor plant. How these abiotic stresses interact with each other and how they effect photosynthesis, plant development, and transpiration are not fully explored in castor (Xue et al. 2017). Among the major nutrients, the availability of N in most soils worldwide is insufficient and is the basic requirement for castor growth. The application of 300 kg ha^{-1} N improved castor yield up to 5939.3 kg ha^{-1} indicating that the higher N the higher the yield of castor (Xue et al. 2017). Nitrogen plays a vital role in the growth of castor as well as other crops, it is involved in protein synthesis, chlorophyll, and metabolism and transformation of energy. Its presence also influences the uptake of some necessary nutrients and contribute to the optimalizing partitioning of photosynthates to reproductive organs increasing the seed (Moro et al. 2012; Torres et al. 2016).

Potassium is involved in maintaining the turgor and cell osmotic balance of plants. Hence its presence affects the development of plants system, mostly the root and shoot system. Indirectly, the availability of potassium is affected by photosynthesis and transcription because cations are involved in the opening and closing of the stoma and cellular turgor (Zubillaga et al. 2002; da Costa et al. 2016). In the process of harvesting of residues, a greater proportion of the potassium remains in the soil with only a small fraction presents in the seed. In contrast to potassium, the phosphorus play a more active action during the period of seed filling (Zubillaga et al. 2002). Phosphorus has been found to help in energy transfer via ATP. It is also a constituent of nucleotides and phospholipids in cell membranes.

The management of some nutrients has been altered in certain areas and growing systems due to abiotic stress and the changing climate (Ramesh et al. 2013). Exogenous application of potassium significantly enhanced castor growth, carboxylation efficiency, carotenoids, chlorophyll, water use efficiency, stomatal conductance, and transpiration (da Costa et al. 2016). It is presupposed that plants suffering from drought stress have a increasing demand for potassium since it is desirable to keep photosynthetic CO_2 fixation (Wang et al. 2013).

Despite the fact that castor plant has high elasticity, its structure is needed to be amended by the presence of nutrients and may affect CO_2 assimilation and light interception, which might not only impair the quality but would also drastically decrease the seed yield.

The NUE of plants can be improved by the creation of proper genotypic variation, methods and selection criteria (Baligar et al. 2001). Selection of cultivars with superior tolerance to suboptimal nutrients levels in soil creates chances of improving the crop production potential on low nutritional lands across the globe (Fageria et al. 2003). In castor breeding programs, the following aspect must be factored: (i) the ability to produce close-maximum yields on soil having low nutrients level (ii) identification of root trait that effectively uses elevated levels of soil nutrients so as to develop cultivars with high NUE that can advance to the sustainability as well as environmental protection.

7.8 Water Use Efficiency

The ratio of total carbon (biomass, photosynthesis, or yield) in plants and the total water loss as a result of transpiration is referred to as water-use efficiency (WUE). The increase in WUE is one strategy to determine plant response to low or high water deficiency in soil, which is in the aim of several researchers that focus to improve plants' tolerance to drought. WUE is also a measure to access water-use strategies in plant and adjustability to various climatic conditions (Lima et al. 2013). Castor is an oilseed plant that requires moderate amount of water and has the capacity to tolerate drought to a certain extent, and with low water intake has moderate yield reduction

(Bhunia et al. 2012). This is due to the deep root system which enables it to draw water from the deeper layers of the soil, and its ability to control water loss from the leaves in the case of water deficit. Nevertheless, long period of water deficiency as a result of climate change can adversely influence the growth and seed oil content of castor plant (Koutroubas et al. 2000).

Water requirements firstly before flowering phase (pre-anthesis) and after flowering (post-anthesis) is crucial to increase castor seed oil yield and limited amount of water supply results in small leaf area index and withered leaves. Though water shortage affects the whole developmental phases of castor, the higher yield reduction takes place in drought during the reproductive phase and at seed filling (Rajala et al. 2011; Severino and Auld 2013b). Castor water requirements vary from 188 to 1178 mm with negligible influence from the environment and soil type (Patel et al. 1998; Severino and Auld 2013b). Ramanjaneyulu et al. (2013) researched irrigation regime on castor oil quality and seed yield during post rainy season in India and found that maximal seed yield (2.13 t ha^{-1}) can be obtained by irrigation. This increase in yield was due to the increasing number of capsules and racemes but not with seed weight (Arnaud 1990; Laureti and Marras 1995).

Agronomical practices such as irrigation is an appropriate method to solve water deficit in plants. Crop rotation, cultivation technology, mulching to decrease soil evaporation, and weed control are some other practices that can be employed but one major setback is their high production costs. Changes in plant morphology, harvest index, leaf hydraulics, root system, and seed properties could solve the issue of water deficit in plant and increase WUE. Therefore, breeding of genotypes with high WUE is most suitable and economical approach to overcome the issue of water deficit (Lakshmamma et al. 2010; Thatikunta et al. 2016).

The presence of genetic variability is a prerequisite to increase WUE in castor plant. Evaluation of 64 germplasm lines of castor showed that lines with significant positive relationship with total dry matter and root characters had high WUE (Lakshmamma et al. 2010). Among the three castor hybrids, Pronto demonstrated as a high WUE hybrid with the highest yield content compare to HD912 and Negus (Koutroubas et al. 2000). Severino and Auld (Severino and Auld 2013b) studied six different cultivars of castor under irrigation and rainfed and found that only cv. BRS Nordestina had the highest yield and tolerance to the amount of water in the soil. Thatikunta et al. (Thatikunta et al. 2016) found that genotypes including PCS 171, PCS-230, RG-48, Kranthi, and SKI-215 were genotypes with superior extrinsic and intrinsic WUE among the 35 castor genotypes that were studied. The above genotypes also had high total dry matter, greening index, and low specific leaf area.

Identification for high WUE genotypes can also be achieved by carbon isotope discrimination (CID) which is very significant in castor breeding designed at creating drought-tolerant genotypes. The lowest value for $\Delta^{13}C$ indicates plant with the highest WUE. This approach gives a long-term measure of WUE unlike other approach like the measurements of gas exchange based on single leaves. Stomatal closure decreases CO_2 and transpiration in leaves under water-stressed conditions. This enforces the enzyme ribulose-1, 5-bisphosphate carboxylase oxygenase (RUBISCO) to discriminate against ^{12}C, which leads to increase in ^{13}C

(El-Soda et al. 2014). In that same study, genotypes including PCS-330, Kranthi, PCS-230, and PCS-265 with low discrimination to $\Delta^{13}C$ had the highest capacity for WUE (Thatikunta et al. 2016).

7.9 Other Abiotic Stresses

7.9.1 Salinity

Salinity, as part of the known abiotic stresses, affects almost 7% of the global land area, furtherly decreasing the productivity of crop (Li et al. 2012). According to the FAO report in 2008, it is supposed that the land area affected by salinity is over 830 million hectares. The presence of saline water leads to salinization of almost 403 million hectares and the activities related with the availability of disproportionately large amount of sodium leads to that of 434 million hectares (FAO 2008). All of these areas include both salt- and drought-affected sites that are unfit for agricultural purposes. Since most arable land for farming is adversely flooded by saline water, the need to sustain food supply for surging population has become a major concern. In view of this, growing of salt-tolerant crops is imperative. Castor plants can grow and survive in marginal lands with with great variation of external salinities compare to other crops (Oleiwi et al. 2016). The exposure of castor plant to 160 mol m^{-3} NaCl grew perfectly well without affecting the seed yield (Jeschke and Wolf 1988). At the early stages of development, castor plant is sensitive to Na, therefore high concentration of salinity stress either by irrigation water or in the soil can inhibit plant growth and productivity (Pinheiro et al. 2008; Silva et al. 2008). The total emergence of castor seed may be delayed and reduced when cultivated in increasing amount of salinity, however significant differences exist between some genotypes (Silva et al. 2005).

It was observed that the photosynthetic apparatus in castor plants was induced upon increasing amount of Na salinity, so was the proline accumulation (Li et al. 2010). The threshold of growth and emergence grown for castor in Na salinity is 7.1 dS $m{-}^{1}$, and there was no significant change in nutrient uptake and accumulation in this elevated level of salt stress (Li et al. 2010). At a salinity of 13.6 dS m^{-1}, the seedling emergence was delayed by 9 days, and the emergence rate was lowered by 50%. If this concentration persists for 11 days, 60% of the seedlings did not survive (Zhou et al. 2010).

High saline stress muddles up ion balance that leads to osmotic stress and ion toxicity and consequently affects plant growth and development (Kumar et al. 2013). The saline stress induces hyperosmotic and hyperionic effects, and disturbs plants' roots from absorbing water, while their maximum levels become toxic inside the plant (Kumar et al. 2013). High salt concentration adversely affected the growth of castor, despite the cationic composition of water. Among the ions studied in the irrigation water, the plant was highly sensitive to the available Na, and Ca and Mg could not mitigate the Na toxic effect during the emergence stage and at the initial growth of castor (Severino et al. 2014; Lima et al. 2018). Comparatively, the effect of the cationic nature in the irrigation water on the emergence and growth of castor was lesser than that of the electrical conductivity, and the negative effects of the cations in the irrigation water followed a trend of $Na^+ > Na^+ + Ca^{2+} > Ca^{2+} > Na^+ + Ca^{2+} + Mg^{2+} > K^+$. The opening of flower buds and time interval for inflorescence development was more influenced by the cationic composition in the irrigation water, and plants irrigated with calcic water demonstrated more of the effects (de Lima et al. 2016).

In plants, elevated levels of saline stress can hinder key biochemical and physiological processes as well as reactive oxygen species hormones (Yan et al. 2017). One primary hormone responsible for stress responses in plants is gibberellic acid (GA_3), and it has been found that presoaking of castor seed with 250 μM GA_3 increases the seedling growth even in maximum amount of saline stress (Jiao et al. 2019). In addition, in order to combat salt stress, some evolutionary strategies such as compartmentalization, which can maintain low cytosolic balance by excluding sodium, and secretion are employed by plants (Purty et al. 2008; Zhang et al. 2014b). Hence, these strategies can be explored in castor to improve its tolerance to saline stress.

7.9.2 Metal Tolerance

Increased industrialization and urbanization have tremendously influenced the dischsrge of higher concentrations of heavy metals, such as cadmium, lead, and nickel into the ecosystem. These heavy metals are highly toxic and non-biodegradable, which can cause much effects to plants and to animals and humans by extension, which have become a major concern worldwide (Jha et al. 2017). It seems that the inherent ability for plants to tolerate and accumulate heavy metal in the soil is the most cost-effective and sustainable remedy.

Castor bann is extensively researched for remediation of metal soils due to its ability to tolerate heavy metals in contaminated soils, because of its large biomass content and high heavy metal accumulating capacity (Jha et al. 2017). Mostly, high accumulation of heavy metals occurs in the roots of castor plant followed by the stem and the leaves (Bauddh and Singh 2012; Çelik and Akdaş 2019). The high accumulation of metals in the roots might be due to the formation of metal complexes which prevent the translocation of the metal from affecting the overall growth and physiology of the plant (Yeboah et al. 2020). Also, the higher amount of metals in plant roots suggests their potential role in stabilizing metal soils (Olivares et al. 2013), hence the ability of castor plants to accumulate heavy metal ions highly in roots proposes it as a potential player in phytostabilization.

The large biomass production of castor plant enhances its tolerance to heavy metal stress. However, to certain extent, the metal stress tends to decrease the plant biomass. The intensity and severity of the metal effect on castor biomass is dependent on the type and concentration of metal stress and duration of metal applied (Bauddh and

Singh 2012; Olivares et al. 2013; Çelik and Akdaş 2019). A pot experiment that examined castor tolerance under Cd, Cu, and Zn-contaminated soils for 4 months showed that the plant had a high biomass level with an average height of 136 cm (Wang et al. 2016). Comparing to the control, castor biomass in Cd-contaminated soil (40 mg/kg) and Cu-contaminated soil (200 and 600 mg/kg) increased by.

−22.9%, 116.9% and −92% respectively. Likewise, biomass under Zn-contaminated soil increased 113.6% and -85% at 200 and 800 mg/kg (Wang et al. 2016). Elevated levels of Cd with 16 mg/L and Pb with 96 mg/L respectively decreased and increased both root and shoot biomass of castor in a hydroponic system. It was concluded that in Cd/Pb contaminated soils, castor plant could be used as an indicator for Cd soil and tolerance for Pb (Costa et al. 2012).

Castor plant employs different mechanisms to tolerate elevated levels of heavy metal stress in the growth medium. These mechanisms include accumulation of proline, compartmentalization, production of antioxidant, phytochelatin, and organic acid secretion (Huang et al. 2016; Nascimento and Marques 2018; Çelik and Akdaş 2019; Ye et al. 2018; Roychowdhury et al. 2019). These mechanisms are effective to enhance the growth of castor to overcome metal stress.

Under heavy metal stress, different antioxidant systems are involved to enhance castor tolerance. The presence of GSH (Glutathione), POD (Peroxidase), and SOD (Superoxide dismutase) activities in the roots and leaf of castor cultivars Zibo No. 8 and Zibo No. 5 promoted its growth upon Cd stress. GSH activity significantly ($p < 0.05$) increased in the root of Zibo No. 8 than in Zibo No. 5 due to higher amount of Cd in the growth medium. This shows that, regardless of the high accumulation of metals in roots, the roots exhibit an effective system that eliminates the metal, an instance observed by the upregulation of antioxidants in the root of Zibo No. 8 (Zhang et al. 2014a). Activities of APX (Ascorbate peroxidase), CAT (Catalase), and PDX (Ascorbate peroxidase) enzymes also significantly increased in castor plants intercropped with alfalfa under Cd stress (Xiong et al. 2018).

Castor could tolerate metal stress by accumulation of proline. Proline, a stress metabolite, serves as a cell wall plasticizer and osmoprotectant which maintain cell membrane and preserves plant cells from dehydrating (Singh et al. 2016). Its expression in castor demonstrates a positive relationship with the stress intensity. Elevated levels of Pb (up to 400 μM) has no effect on castor due to its proline content (Kiran and Prasad 2017). There was a rise in osmoprotectant proline in the leaves of castor when cultivated in Ni-contaminated soil and was concluded that the increase of proline under Ni stress depends on plant species and concentration of the metal toxicity (Bauddh and Singh 2015).

Compartmentalization is also one of the effective strategy castor plant exhibits to combat elevated levels of metal stress without the plant showing any toxic symptoms. To achieve detoxification, the plant either transport the metal ions out of the cell or sequestrate in cell wall or vacuole to limit the metal effect on other sensitive metabolic activities in the cell. A number of studies have reported metal accumulation in the roots, stems, and leaves of castor in the growth medium (Nascimento and Marques 2018). Metal transporters such as ABC transporter protein enhances castor to sequester metal ions in the subcellular compartment (Pal et al. 2013). The subcellular partitioning which includes cellular debris, organelle, heat-stable protein (HSP), metal-rich granules (MRG), and heat denature proteins (HDP) are classified based on the differential centrifugation of tissues and this partitioning gives an idea of metal tolerance in plants (Zhang et al. 2015). Castor plant exposure to 2 and 5 mg/L Cd was more pronounced in the order of soluble fraction, MRG, and organelles (Zhang et al. 2015). In addition, low secretion of organic acids from the roots of castor plant enhanced its growth in heavy metal contaminated soils. Organic acids such as citric acid and malate acid increased in the roots in Ni-contaminated soil (Bauddh and Singh 2015), and tartaric acid, low cysteine, and oxalic acid-enhanced its growth upon Cu stress with 750 μmol/L (Huang et al. 2016). Lastly, high-affinity ligands such as phytochelatins promote castor plants to alleviate metal stress especially in Ni-contaminated soil (Adhikari and Kumar 2012).

7.9.3 Photoperiod

Castor originated in low latitudes, and its photoperiod sensitivity varied significantly among genotypes. The photoperiod of materials from and around the equator is the most sensitive, which are difficult or not to bloom when introduced to other regions. But there are photoperiod insensitive types in castor. There are many wild castor plants with photoperiod insensitivity in South China. Sowing in different periods has great influence on growth period and yield. Taking South China as an example, many of the materials seeded in February bloomed in less than a month, and the main ears were small, however, the spikes on secondary and tertiary branches can grow very large. Some varieties can blossom if sown in autumn, but can not blossom when sown in spring. Photoperiod insensitivity is important for variety adaptability, at the same time, it is very important to achieve high yield through reasonable sowing date and proper yield structure.

7.10 Traditional Breeding Methods

Castor has relatively good tolerance and adaptability to different abiotic stresses. High tolerance and strong adaptability can be selected by germplasm resource identification and screening, systematic breeding and hybrid breeding. For example, the tolerance and accumulation of castor to heavy metals are significantly different between different genotypes. Therefore, the tolerance genotype can be screened under certain concentration stress. The main screening indexes include germination rate, seedling emergence rate, biomass and accumulation rate. Unfortunately, the progress of molecular breeding under abiotic stress in castor is slow, with slight molecular or morphological markers reported. Because the genetic mechanism of castor biological stress is little known, its genome design and breeding is still in its infancy. With the publication of high quality genome sequences and the progress of genetic research, castor breeding against abiotic stress is about to usher in a bright future.

References

Adhikari T, Kumar A (2012) Phytoaccumulation and tolerance of *Riccinus communis* L. to nickel. Intl J Phytorem 14:481–492

Aouass K, kenny L, Krim J (2020) Nitrogen use efficiency in organic melon production under greenhouse conditions in South West of Morocco. Moroccan J Agric Sci 2550:553X

Arnaud F (1990) The development of castor-oil crops in France, Il ricino: obiettivi, strategie e ricerca. Agricoltura Ricerca. Ministero agricoltura e foreste Roma

Babita M, Maheswari M, Rao L, Shanker AK, Rao DG (2010) Osmotic adjustment, drought tolerance and yield in castor (*Ricinus communis* L.) hybrids. Environ Exp Bot 69:243–249

Baldwin BS, Cossar RD (2009) Castor yield in response to planting date at four locations in the south-central United States. Ind Crops Prod 29:316–319

Baligar V, Fageria N, He Z (2001) Nutrient use efficiency in plants. Commun Soil Sci Plan 32:921–950

Baligar VC, Fageria NK (2015) Nutrient use efficiency in plants: an overview. In: Rakshit A, Singh HB, Sen A (eds) Nutrient use efficiency: from basics to advances. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2169-2_1

Bauddh K, Singh RP (2012) Growth, tolerance efficiency and phytoremediation potential of *Ricinus communis* (L.) and *Brassica juncea* (L.) in salinity and drought affected cadmium contaminated soil. Ecotoxicol Environ Saf 85:13–22

Bauddh K, Singh RP (2015) Assessment of metal uptake capacity of castor bean and mustard for phytoremediation of nickel from contaminated soil. Bioremed J 19:124–138

Beltrão N, Souza J, Santos J (2006) Metabolic alterations happened in castor (BRS 149-Nordestina) due to the hydric stress for deficiency and excess in the soil. Rev Bras De Oleaginosas e Fibrosas 10:977–984

Bhunia S, Chauhan R, Yadav B (2012) Effect of plant geometry and irrigation levels on productivity, yield components, water use and water use efficiency of castor (*Ricinus communis*) in canal command areas of north-western Rajasthan. J Rural Agric Res 12:37–39

Blum A (2017) Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant Cell Environ 40:4–10

Çelik Ö, Akdaş EY (2019) Tissue-specific transcriptional regulation of seven heavy metal stress-responsive miRNAs and their putative targets in nickel indicator castor bean (*R. communis* L.) plants. Ecotoxicol Environ Saf 170:682–690

Costa dSET, Guilherme LRG, de Melo ÉEC, Ribeiro BT, Euzelina dos Santos BI, da Costa Severiano E, Faquin V, Hale BA (2012) Assessing the tolerance of castor bean to Cd and Pb for phytoremediation purposes. Biol Trace Elem Re 145:93–100.

da Costa JCF, Júnior GSC, de Lourdes R, de Lima S, Gheyi HR, Sofiatti V, Soares MRA (2016) Gas exchange in castor bean cultivars in response to foliar application of potassium silicate. Afr J Agric Res 11:4077–4085

de Lima GS, Gheyi HR, Nobre RG, Xavier DA, dos Anjos Soares LA, Cavalcante LF, dos Santos JB (2016) Emergence, growth, and flowering of castor beans as a function of the cationic composition of irrigation water. Semi Ciências Agrárias 37:651–664

Else MA, Coupland D, Dutton L, Jackson MB (2001) Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (*Ricinus communis*) despite diminished delivery of ABA from the roots to shoots in xylem sap. Physiol Planta 111:46–54

El-Soda M, Malosetti M, Zwaan BJ, Koornneef M, Aarts MG (2014) Genotype × environment interaction QTL mapping in plants: lessons from Arabidopsis. Trends Plant Sci 19:390–398

Fageria N, Slaton N, Baligar V (2003) Nutrient management for improving lowland rice productivity and sustainability. Adv Agron 80:63–152

FAO (2008) FAO land and plant nutrition management service. Available at: http://www.fao.org/ag/agl/agll/spush

Fukao T, Barrera-Figueroa BE, Juntawong P, Peña-Castro JM (2019) Submergence and waterlogging stress in plants: a review highlighting research opportunities and understudied aspects. Front Plant Sci 10:340

Funk JL, Zachary VA (2010) Physiological responses to short-term water and light stress in native and invasive plant species in southern California. Biol Invas 12:1685–1694

Hasanuzzaman M, Nahar K, Alam M, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Intl J Mol Sci 14:9643–9684

Huang G, Guo G, Yao S, Zhang N, Hu H (2016) Organic acids, amino acids compositions in the root exudates and Cu-accumulation in castor (*Ricinus communis* L.) under Cu stress. Intl J Phytorem 18:33–40

Jeschke WD, Wolf O (1988) Effect of NaCI salinity on growth, development, ion distribution, and ion translocation in castor bean (*Ricinus communis* L.). J Plant Physiol 132:45–53

Jha AB, Misra AN, Sharma P (2017) Phytoremediation of heavy metal-contaminated soil using bioenergy crops. In: Bauddh K, Singh B, Korstad J (eds) Phytoremediation potential of bioenergy plants. Springer, pp 63–96

Jiao X, Zhi W, Liu G, Zhu G, Feng G, Eltyb Ahmed Nimir N, Ahmad I, Zhou G (2019) Responses of foreign GA3 application on seedling growth of castor bean (*Ricinus communis* L.) under salinity stress conditions. Agronomy 9:274

Kiran BR, Prasad MNV (2017) Responses of *Ricinus communis* L. (castor bean, phytoremediation crop) seedlings to lead (Pb) toxicity in hydroponics. Selcuk J Agri Food Sci 31:73–80

Kolaksazov M, Laporte F, Ananieva K, Dobrev P, Herzog M, Ananiev E (2013) Effect of chilling and freezing stresses on jasmonate content in Arabis alpina. Bulg J Agric Sci 19:15–17

Koutroubas S, Papakosta, D Doitsinis A (2000) Water requirements for castor oil crop (*Ricinus communis* L.) in a Mediterranean climate. J Agron Crop Sci 184:33-41

Kumar K, Kumar M, Kim S-R, Ryu H, Cho Y-G (2013) Insights into genomics of salt stress response in rice. Rice 6:1–15

Lakshmamma P, Prayaga L, Sarada C (2010) Evaluation of castor (*Ricinus communis* L.) germplasm for water use efficiency (WUE) and root characters. Indian J Plant Genet Resour 23:276–279

Lakshmamma P, Lakshminarayana M, Prayaga L, Alivelu K, Lavanya C (2009) Effect of defoliation on seed yield of castor (*Ricinus communis* L.). Indian J Agric Sci 79:630–623

Laureti D, Marras G (1995) Irrigation of castor (*Ricinus communis* L.) in Italy. EurJ Agron 4:229–235

Li G, Wan S, Zhou J, Yang Z, Qin P (2010) Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. Ind Crops Prod 31:13–19

Li J-g, Pu L-j, Zhu M, Zhang R (2012) The present situation and hot issues in the salt-affected soil research. Acta Geogr Sin 67:1233–1245

Lima JRdS, Antonino ACD, Souza ESd, Lira CABdO, Silva IdFd (2013) Seasonal and interannual variations of evapotranspiration, energy exchange, yield and water use efficiency of castor grown under rainfed conditions in northeastern Brazil. Ind Crops Prod 50:203–211

Lima GSd, Nobre RG, Gheyi HR, Soares LAdA, de Azevedo CA, de Lima VL (2018) Salinity and cationic nature of irrigation water on castor bean cultivation. Revista Brasileira de Engenharia Agrícola e Ambiental 22:267–272

Lipiec J, Doussan C, Nosalewicz A, Kondracka K (2013) Effect of drought and heat stresses on plant growth and yield: a review. Intl Agrophys 27

Liu HR, Li FQ, Wu M (2005) Analysis of Agrometeorological conditions and preliminary study on cultivation techniques of castor. Xinjiang Meteorol J 01:16–17

Manjula K, Sarma P, Thatikunta R, Rao TN (2003) Evaluation of castor (*Ricinus Communis* L.) genotypes for moisture stress. Indian J Plant Physiol 8:319–322

Moro E, Crusciol CAC, de Oliveira P, Sipos TB (2012) Nitrogen fertilizers and rates in castor bean hybrids under no-till system/Fontes e doses de nitrogenio para mamoneira de porte baixo no sistema plantio direto. Cienc Rural 42:1744–1752

Moshkin V (1986) Castor. Taylor & Francis: Amerind, New Delhi, India

Naik B (2018) Botanical descriptions of castor bean. In: Kole C, Rabinowicz P (eds) The castor bean genome. Springer, pp 1–14

Nascimento CWAd, Marques MC (2018) Metabolic alterations and X-ray chlorophyll fluorescence for the early detection of lead stress in castor bean (*Ricinus communis*) plants. Acta Sci Agron 40:2–9

Oleiwi AM, Elsahookie MM, Mohammed LI (2016) Performance of castor bean selects in saline sodic soil. Intl J Appl Agric Sci 2:64

Olivares AR, Carrillo-González R, González-Chávez MdCA, Hernández RMS (2013) Potential of castor bean (*Ricinus communis* L.) for phytoremediation of mine tailings and oil production. J Environ Manag 114:316–323

Pal R, Banerjee A, Kundu R (2013) Responses of castor bean (*Ricinus communis* L.) to lead stress. Proc Natl Acad Sci India Sect B Biol Sci 83:643–650

Panjabi P, Yadava SK, Kumar N, Bangkim R, Ramchiary N (2019) Breeding brassica juncea and B. rapa for sustainable oilseed production in the changing climate: progress and prospects. In: Kole C (ed) Genomic designing of climate-smart oilseed crops. Springer, Cham, Switzerland AG, pp 275–369

Patel B, Maliwal G, Dudhatra N, Kaswala R, Patel P, Raman S, Patel M (1998) Effect of drip irrigation on yield and water expense efficiency of castor (*Ricinus communis* L.). Gujarat Agric Univ Resh J (india) 24:86–88

Patel RR, Patel DD, Thakor P, Patel B, Thakkar VR (2015) Alleviation of salt stress in germination of Vigna radiata L. by two halotolerant Bacilli sp. isolated from saline habitats of Gujarat. Plant Growth Regul 76:51–60

Pinheiro HA, Silva JV, Endres L, Ferreira VM, de Albuquerque CC, Cabral FF, Oliveira JF, de Carvalho LWT, dos Santos JM, dos Santos FBG (2008) Leaf gas exchange, chloroplastic pigments and dry matter accumulation in castor bean (*Ricinuscommunis* L) seedlings subjected to salt stress conditions. Ind Crops Prod 27:385–392

Purty RS, Kumar G, Singla-Pareek SL, Pareek A (2008) Towards salinity tolerance in Brassica: an overview. Physiol Mol Biol Plants 14:39–49

Rajala A, Hakala K, Mäkelä P, Peltonen-Sainio P (2011) Drought effect on grain number and grain weight at spike and spikelet level in six-row spring barley. J Agron Crop Sci 197:103–112

Ramanjaneyulu A, Reddy AV, Madhavi A (2013) The impact of sowing date and irrigation regime on castor (*Ricinus communis* L.) seed yield, oil quality characteristics and fatty acid composition during post rainy season in South India. Ind Crops Prod 44:25–31

Ramesh P, Babu S, Qureshi MAA, Rao TN (2013) Effect of conservation agricultural and nutrient management practices on castor (*Ricinus communis*)–sorghum (Sorghum bicolor) cropping system in rainfed Alfisols. Indian J Agron 58:168–174

Roychowdhury R, Roy M, Zaman S, Mitra A (2019) Phytoremediation potential ofcastor oil plant (*Ricinus Communis*) grown on fly ash amended soil towards lead bioaccumulation. J Emerg Technol Innov Res (JETIR) 6:156–160

Sala CA, Bulos M, Altieri E, Ramos ML (2012) Sunflower: improving crop productivity and abiotic stress tolerance. In: Tuteja N, Gill SS, Tiburcio AF, Tuteja R (eds) Improving crop resistance to abiotic stress. Wiley-VCH, pp 1203–1249

Salihu B, Gana A, Apuyor B (2014) Castor oil plant (*Ricinus communis* L.): botany, ecology and uses. Intl J Sci Res 3:1334–1341

Sausen TL, Rosa LMG (2010) Growth and carbon assimilation limitations in *Ricinus communis* (Euphorbiaceae) under soil water stress conditions. Acta Bot Brasil 24:648–654

Severino LS, Auld DL (2013a) A framework for the study of the growth and development of castor plant. Ind Crops Prod 46:25–38

Severino LS, Auld DL (2013b) Seed yield and yield components of castor influenced by irrigation. Ind Crops Prod 49:52–60

Severino LS, Lima RL, Castillo N, Lucena AM, Auld DL, Udeigwe TK (2014) Calcium and magnesium do not alleviate the toxic effect of sodium on the emergence and initial growth of castor, cotton, and safflower. Ind Crops Prod 57:90–97

Severino L, Beltrão NdM, Carsoso G, Farias VdA (2005) Comportamento da mamoneira sob encharcamento do solo. Boletim de Pesquisa 57:1–14

Shanker AK, Maheswari M, Yadav S, Desai S, Bhanu D, Attal NB, Venkateswarlu B (2014) Drought stress responses in crops. Funct Integr Genom 14:11–22

Silva S, Alves A, Ghey H, Beltrão N, Severino L, Soares F (2005) Germination and initial growth of two cultivars of castor bean under saline stress. (Portuguese, with English abstract). Rev Bras de Engenharia Agrícola e Ambiental Suppl 347–352

Silva S, Alves A, Ghey H, Beltrão NdM, Severino L, Soares F (2008) Growth and production of two cultivars of castor bean under saline stress. (Portuguese, with English abstract). Rev Bras de Engenharia Agrícola e Ambiental 12:335–342

Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front Plant Sci 6:1143

Škorić D (2016) Sunflower breeding for resistance to abiotic and biotic stresses. In: Shanker AK Shanker C (eds) Abiotic and biotic stress in plants—recent advances and future perspectives. IntechOpen, London, pp 585–635

Thatikunta R, Sankar AS, Sagarika L, Palle G, Sreelakshmi J, Shankar VG, Rani CVD, Lavanya B (2016) Identification of genotypes for high water use efficiency and root traits in castor (*Ricinus communis* L.). Indian Soc Oilseed Res 33:87–90

Torres FE, Teodoro PE, Hernandes FB, Fernandes RL, Gomes AC, Lopes KV (2016) Agronomic performance of castor under different growing conditions. Biosci J 32:55–60

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223

Wang M, Zheng Q, Shen Q, Guo S (2013) The critical role of potassium in plant stress response. Intl J Mol Sci 14:7370–7390

Wang S, Zhao Y, Guo J, Zhou L (2016) Effects of Cd, Cu and Zn on *Ricinus communis* L. growth in single element or co-contaminated soils: pot experiments. Ecol Engg 90:347–351

Wilson CO, Liang B, Rose SJ (2019) Projecting future land use/land cover by integrating drivers and plan prescriptions: the case for watershed applications. Gisci Remote Sens 56:511–535

Xiong P-p, He C-q, Oh K, Chen X, Liang X, Liu X, Cheng X, Wu C-l, Shi Z-c (2018) Medicago sativa L. enhances the phytoextraction of cadmium and zinc by *Ricinus communis* L. on contaminated land in situ. Ecol Engg 116:61–66

Xue X, Mai W, Zhao Z, Zhang K, Tian C (2017) Optimized nitrogen fertilizer application enhances absorption of soil nitrogen and yield of castor with drip irrigation under mulch film. Ind Crops Prod 95:156–162

Yan K, Zhao S, Bian L, Chen X (2017) Saline stress enhanced accumulation of leaf phenolics in honeysuckle (*Lonicera japonica* Thunb.) without induction of oxidative stress. Plant Physiol Biochem 112:326–334

Ye W, Guo G, Wu F, Fan T, Lu H, Chen H, Li X, Ma Y (2018) Absorption, translocation, and detoxification of Cd in two different castor bean (*Ricinus communis* L.) cultivars. Environ Sci Pollut Res 25:28899–28906

Yeboah A, Lu J, Yang T, Shi Y, Amoanimaa-Dede H, Agyenim-Boateng KG, Yin X (2020) Assessment of castor plant (*Ricinus communis* L.) tolerance to heavy metal stress. Phyton Intl J Exp Bot 89:1–20

Yin X, Lu J, Agyenim-Boateng KG, Liu S (2019) Breeding for climate resilience in castor: Current status, challenges, and opportunities. In: Kole C (ed) Genomic designing of climate-smart oilseed crops. Springer, Cham, pp 441–498

Zhang H, Guo Q, Yang J, Chen T, Zhu G, Peters M, Wei R, Tian L, Wang C, Tan D, Ma J, Wang G, Wan Y (2014a) Cadmium accumulation and tolerance of two castor cultivars in relation to antioxidant systems. J Environ Sci 26:2048–2055

Zhang X, Lu G, Long W, Zou X, Li F, Nishio T (2014b) Recent progress in drought and salt tolerance studies in Brassica crops. Breed Sci 64:60–73

Zhang H, Guo Q, Yang J, Shen J, Chen T, Zhu G, Chen H, Shao C (2015) Subcellular cadmium distribution and antioxidant enzymatic activities in the leaves of two castor (*Ricinus communis* L.) cultivars exhibit differences in Cd accumulation. Ecotoxicol Environ Saf 120:184–192

Zhong W, Hartung W, Komor E, Schobert C (1996) Phloem transport of abscisic acid in *Ricinus communis* L. seedlings. Plant Cell Environ 19:471–477

Zhou G, Ma B, Li J, Feng C, Lu J, Qin P (2010) Determining salinity threshold level for castor bean emergence and stand establishment. Crop Sci 50:2030–2036

Zubillaga M, Aristi J, Lavado R (2002) Effect of phosphorus and nitrogen fertilization on sunflower (*Helianthus annus* L.) nitrogen uptake and yield. J Agron Crop Sci 188:267–274

Chapter 8
Designing Genomic Solutions to Enhance Abiotic Stress Resistance in Flax

Nadeem Khan, Frank M. You, and Sylvie Cloutier

Abstract Flax is a self-pollinated crop widely cultivated for fiber and oilseed production. Poor rooting system architecture and abiotic stresses such as drought, extreme temperature, salt, and cadmium accumulation can significantly affect the growth of flax, resulting in severe production losses. As such, improving the root system of flax cultivars and increasing their resistance or tolerance to abiotic stresses are important to achieve sustainable flax production. In recent years, substantial progress has been made towards the generation of flax genomic resources. Reduced cost of high-throughput sequencing has fueled the large-scale production of sequence data, allowing an intensification in the genotyping of numerous populations for quantitative trait locus (QTL) mapping. Advanced genome-wide association studies (GWAS) combined with candidate gene identification through bioinformatics approaches make it possible to identify many large- and small-effect quantitative trait loci (QTLs) and candidate genes associated with agronomically important traits. To date, a total of 521 QTLs associated with abiotic stress-related traits have been identified in flax. These QTLs constitute markers for genomic selection (GS) to predict breeding values of populations under selection; the goals being to improve the accuracy and efficiency of selection, reduce cost, and shorten the breeding cycles. Combined with genetic simulation, this GS strategy offers a new, effective approach to predict the breeding performance of crosses and evaluate parents based on their genotype. Here we provide an overview of genomewide QTL mapping, gene family identification, and outline the potential of a combined GS strategy with genetic simulation for breeding improvement of abiotic stress tolerance in flax.

N. Khan · F. M. You (✉) · S. Cloutier (✉)
Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON K1OC6, Canada
e-mail: frank.you@agr.gc.ca

S. Cloutier
e-mail: sylviej.cloutier@agr.gc.ca

N. Khan
e-mail: nadeem.khan@agr.gc.ca

N. Khan · S. Cloutier
Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON K1N 6N5, Canada

C. Kole (ed.), *Genomic Designing for Abiotic Stress Resistant Oilseed Crops*,
https://doi.org/10.1007/978-3-030-90044-1_8

Keywords Flax · Genomewide markers · Quantitative trait loci (QTL) · Genomewide association studies (GWAS) · Genomic selection (GS) · Genetic simulation · Abiotic stress

8.1 Introduction

Stress in plants is defined as external conditions that negatively disturb plant growth and productivity, mainly as a consequence of their sessile nature (Zhu 2016). A plant must cope with adverse circumstances such as drought, heat, cold, salt, and metal toxicity. Collectively, these stresses are known as abiotic stresses, and they pose a great threat to worldwide agricultural productivity (Wang et al. 2004; Wani et al. 2016). Understanding abiotic stress coping mechanisms in plants will lead to the design of counteracting strategies that ultimately aim to ensure food security and agricultural sustainability for a growing world population. Global temperatures are expected to increase approximately 0.2 °C per decade over the next thirty years, and this rise in temperature is forecasted to affect crop productivity (Bailey-Serres et al. 2019). Reduction in freshwater availability and shrinking of biodiversity have already altered crop growth as exemplified by yield reduction in affected regions (Keesing et al. 2010; Brown et al. 2019). Such changes are not uniformly distributed around the world. For instance, the European Mediterranean countries are expected to experience warmer temperatures with regular drought stress (DS), while temperature increases in North America is predicted to affect the cycle of the rainy season from spring to winter (Hopkin 2005). Climatic changes such as heat stress (HS) can lead to plant death. One study showed that HS and DS caused up to a 6.2% reduction in cereal production between 2000 and 2007 (Lesk et al. 2016). Paradoxically, as a result of the global rise in temperature, excessive amounts of rainfall are also a major threat for crop production. For example, yield losses in maize totaling US$ 10B from 1989 to 2016 were due to heavy rainfall, a figure of comparable magnitude to the losses caused by extreme drought in the USA (Li et al. 2019b). Salt stress (SS) is also a growing threat to plant growth because of the increasing amount of salinization worldwide (Munns and Tester 2008). In high salt concentration, i.e., 200 mM, most plants are unable to survive (Flowers 2008; Zhou et al. 2016). High salinity leads to an increase in ionic toxicity, an increase in osmotic pressure, and disturbs growth from seedling to flowering (Zhao et al. 2010; Feng et al. 2014; Guo et al. 2018). SS and DS are often associated, thereby compounding the challenge (Ashraf and Foolad 2007; Slama et al. 2015). Further, heavy metal (HM) stress has also become a concern worldwide due to extensive industrialization and because it directly and indirectly affects soil health and crop productivity (Shahid et al. 2015). HMs can cause significant yield losses and disturb various physiological and molecular responses in plants (Panuccio et al. 2009; Hassan et al. 2017). Most HMs, for example zinc, copper, manganese, cobalt, and nickel, are crucial for various biological processes (Salla et al. 2011). In contrast, arsenic, lead, cadmium (Cd), among others, are highly toxic and adversely affect plant growth and productivity (Xiong et al. 2014;

Pierart et al. 2015). Several studies have identified candidate genes or loci involved in HM response, particularly for Cd stress (Wu et al. 2015; Chen et al. 2018; Zhao et al. 2018). Overall, it is imperative to develop stress resistant cultivars for two main reasons: (i) to respond to the global environmental changes, and (ii) to meet the global demand for healthy food.

Flax (*Linum usitatissimum* L.) is a self-pollinated crop that is widely grown for fiber and oilseed production. It is a tap-rooted plant with a shallow root system that depends largely on moisture and nutrient resources mostly located in the top 70 cm of soil (Hocking et al. 1997; Kar et al. 2007; Hall et al. 2016). Drought (Soto-Cerda et al. 2019, 2020), extreme temperatures (heat and cold stresses) (Cross et al. 2003; Tchoumtchoua et al. 2019), salt (Hashem et al. 2011; Yu et al. 2014), and mineral toxicity (Angelova et al. 2004) negatively impact the growth and development of flax. To facilitate abiotic stress studies, tremendous progress has been made towards the development of flax genomic resources including the first assembly of the flax reference genome into 15 pseudomolecules, its BioNano optical map, a consensus genetic map, and a first-generation haplotype map constructed using 407 diverse accessions and ~1.7 million single nucleotide polymorphisms (SNPs) (Cloutier et al. 2012; You et al. 2018). These genomic resources lay a solid foundation for flax improvement, including abiotic stress-related traits. Traditional approaches have not been successful because these traits are often controlled by many genes. Identification of key candidate genes through genomewide association studies (GWAS) and other genomewide investigations can be capitalized upon to address the problems associated with abiotic stress tolerance. Several candidate genes have been identified successfully through GWAS, for root traits and DS tolerance (Sertse et al. 2019; Soto-Cerda et al. 2019, 2020). These genes can be pyramided into elite genotypes via marker-assisted selection to produce abiotic stress tolerant cultivars.

This review summarizes the outcomes of genomic studies and their potential uses in mitigating plants' responses to abiotic stresses. First, the importance of GWAS in flax and other species experiencing a variety of abiotic stresses is discussed prior to addressing genomewide strategies for gene family identification. Recent concepts and novel strategies applied to abiotic stresses such as genomic selection (GS) and genetic simulation are outlined in view of their applications in flax breeding. This knowledge holds potential in genomic studies for abiotic stress tolerance, not only in flax but also in other economically important species. Candidate gene mining can and must be performed imminently to benefit the development of abiotic stress resistant or tolerant cultivars in a timely manner and in view of the potential severe consequences associated with climate change.

8.2 Genomics for Crop Improvement

Rapid advances in genomics and the availability of inexpensive and reliable high-throughput sequencing (HTS) technologies make it possible to sequence billions of fragments of DNA sequences. Nowadays, short- and long-read sequence technologies are amenable to a wide range of genomes compared to first-generation sequencing (Mardis 2008; Amarasinghe et al. 2020). In modern crop genomics, DNA markers such as SNPs are broadly used because of their high polymorphism, ubiquitous nature, and low cost. Large sets of genotypes with thousands to millions of markers are routinely produced in several plant species. Further, whole-genome resequencing data is gaining popularity and provides insights into structural diversity (Voss-Fels and Snowdon 2016). The recent development in HTS and the use of genomic approaches will provide key strategies to promote efficiency and precision in flax breeding research including studies of abiotic stress tolerant and sensitive genotypes. For instance, the reference genome sequence of flax and its first-generation haplotype map comprising 407 diverse accessions and ~1.7 million SNPs have recently been updated (You et al. 2018; Wang et al. 2019a). A better understanding of fundamental molecular information has the potential to improve flax breeding practices and accuracy towards superior abiotic stress tolerance. Finding the relationship between genotypic and phenotypic variations using GWAS can provide insights to assist flax breeding programs in designing the most efficient breeding strategies. Once improved, genotypes adapted to respond to abiotic stresses like drought, heat, and metal toxicity for instance, offer not only a practical genetic improvement but also a sustainable solution towards bridging the food security gap. Currently, yield improvement or stability of crops exposed to abiotic stresses are major goals of plant breeding programs in many crops worldwide (Ciesla et al. 2016; Landi et al. 2017). Generally, accurate and reliable phenotyping is labor-intensive and time-consuming. The use of high-throughput phenotyping (HTP), GS, and genetic simulation offers alternative ways of tackling this issue to achieve accuracy and precision in breeding. Currently, a combination of abiotic stress-related indices, methods, and population types have been used to identify QTLs and candidate genes including GWAS, genomewide identification of candidate genes, doubled haploids (DHs), near-isogenic lines (NILs), bi-parental population, and others (Khadivi-Khub 2014; Xia et al. 2014; Fu et al. 2017; Khan et al. 2018b; Sukumaran et al. 2018; Wang et al. 2018; Soto-Cerda et al. 2019). Here we will discuss studies focusing on the identification of abiotic stress-related QTL including traits such as root architecture, drought, water efficiency, heat, cold, salt, and Cd stresses, mainly in flax, but also in other crops.

8.2.1 Genome-Wide Association Studies (GWAS)

GWAS aim to establish associations between genotype and phenotype and to quantify the contribution of genetic variants across the genomes of many individuals to the measured traits. They are a powerful tool to predict the candidate genes underlying complex traits (Nicod et al. 2016). GWAS exploit a large set of genetic variants to identify a subset of variants associated with a trait of interest (Tam et al. 2019). They can be based on multiple environments, years, and traits. GWAS have been successful in identifying QTLs for abiotic stress-related traits including root architecture and drought tolerance (Sertse et al. 2019; Soto-Cerda et al. 2019, 2020; Liu et al. 2020;

Yonis et al. 2020), heat and cold tolerances (Chen et al. 2017; Lafarge et al. 2017; Song et al. 2018; Zhang et al. 2018), and Cd stress (Wu et al. 2015; Chen et al. 2018) in flax, as well as in many other plant species. These are summarized in Table 8.1.

The QTLs identified in early studies were mostly obtained using the general linear model (GLM) or the mixed linear model (MLM) which tended to be the main single-locus methods at the time. The power of GLM and MLM lies in its ability to detect large effect quantitative trait nucleotides (QTNs) or QTLs compared to the multi-locus models that were developed later and that are better suited to detect smaller effect QTNs or QTLs. An example of such model is the multi-locus random-SNP-effect mixed linear model (mrMLM) (Wang et al. 2016b; He et al. 2019). In flax, a total of 521 QTLs were identified for root traits and drought stress using a combination of single- and multi-locus models (Sertse et al. 2019, 2021; Soto-Cerda et al. 2019, 2020). Multi- and single-locus models are somewhat complementary and their combined use enables the identification of both small and large effect QTNs for complex and low heritability traits. A common concern in GWAS is the consequent difficulties associated with the identification of causal variants and genes associated with abiotic stress-related traits that have relatively low heritability. Such candidate genes must be further validated through functional genomic experiments. This problem may be overcome by increasing the sample size; however, this would greatly increase the cost of the experiment. The other problem typically associated with GWAS is the elimination of markers with low allele frequency, i.e., markers with alleles present in less than 5–10% of the individuals (Hawkesford and Griffiths 2019). Minor alleles can be difficult to ascertain and the statistical power of detection of association is poor because it is derived from few individuals; hence, the minor allele frequency cut-offs of 5–10%, commonly used depending on the population size, precludes genotype–phenotype associations of these rare alleles. Proper design is critical in GWAS, including accounting for the genetic structure of the population to minimize both false-negative and false-positive results. With proper methodology, GWAS has been applied successfully to identify numerous key genes. For example, many of the genes commonly used by the US Food and Drug Administration as molecular targets in several drugs were identified by GWAS, and this success strongly supports the continuous efforts to improve the methodology and to consider its application to large-size populations (Altshuler et al. 2008; Hirschhorn 2009). This section will summarize several studies focusing strictly on GWAS for abiotic stress-related traits including root architecture, water efficiency, and tolerance to drought, heat, cold, salt, and Cd.

8.2.1.1 Root Characters

The root system is a critical tissue implicated in many abiotic stress responses but current knowledge of its role is limited because its phenotyping is challenging. Consequently, improvement of root architecture is a daunting task that must be overcome to improve plant adaptation against drought stress, water efficiency, nutrient supplies, and others. Because of the inherent difficulties associated with accurate phenotyping

Table 8.1 QTL identification in different species in response to abiotic stresses

Trait	Species	Pop size	No. markers	Method	Statistical model	No. QTL	No. traits	References
Root	Flax	115	7707 & 3243	GWAS	6 multi- & 1 single-locus	228	16	Sertse et al. (2019)
	Barley	221	6336	GWAS	MLM	55	6	Jia et al. (2019)
	Maize	384	681,257	GWAS	LM & MLM	268	22	Pace et al. (2015)
	Maize	297	131,271	GWAS	GLM/MLM	34	13	Wang et al. (2019a)
	Wheat	199	12,109	QTL mapping	CIM	18	8	Li et al. (2019a)
Drought	Flax	115	12,316	GWAS	6 multi- & 1 single-locus	148	11	Sertse et al. (2021)
	Flax	41	170,534	GWAS	3 multi- & 2 single-locus	118	4	Soto-Cerda et al. (2020)
	Flax	105	394	SSA	MRA	27	8	Soto-Cerda et al. (2019)
	Soybean	259	4616	GWAS	MLM	15	3	Liu et al. (2020)
	Chrysanthemum	88	92,811	GWAS	GLM & MLM	137 & 14	7	Su et al. (2019)
	Maize	144	45,868	GWAS	GLM & MLM	47	6	Zhang et al. (2013)
	Wheat	100	15,600	GWAS	MLM	75	6	Mathew et al. (2019)
	Barley	156	4438	GWAS	MLM	14	5	Wehner et al. (2016)
Heat	Sorghum	374	13,987	GWAS	MLM	14	2	Chen et al. (2017)
	Wheat	200	15,574	GWAS	MLM	15	4	Maulana et al. (2018)
	Rice	167	13,160	GWAS	MLM	14	20	Lafarge et al. (2017)
	Rapeseed	88	37,539	GWAS	GLM & MLM	20	3	Rahaman et al. (2018)

(continued)

Table 8.1 (continued)

Trait	Species	Pop size	No. markers	Method	Statistical model	No. QTL	No. traits	References
Cold	Rice	115	67,511	GWAS	MLM	26	5	Song et al. (2018)
	Rice	295	44,100	GWAS	MLM	67	3	Wang et al. (2016a)
	Rice	202	157	GWAS	MLM	48	5	Schläppi et al. (2017)
	Rice	249	3867	GWAS	MLM	47	2	Zhang et al. (2018)
	Maize	306 & 292	49,585	GWAS	MLM	47 & 4	2	Revilla et al. (2016)
	Barley	184	1536	GWAS	MLM	12 & 7	2	Visioni et al. (2013)
Salt	Rice	478	162,529	GWAS	6 multi-locus	378	21	Cui et al. (2018)
	Rice	208	395,553	GWAS	mrMLM	20	11	Naveed et al. (2018)
	Rice	708	1,101,404	GWAS	MLM	2255	8	Liu et al. (2019a)
	Soybean	305	37,573	GWAS	EMMAX & MLMM	29	4	Do et al. (2019)
	Barley	350	~24,000	GWAS	MLM	52	4	Mwando et al. (2020)
	Alfalfa	291	7401	GWAS	MLM	53	5	Liu et al. (2019f)
	Alfalfa	304	6862	GWAS & GP	MLM	27	4	Medina et al. (2020)
Cadmium	Barley	100	1536	GWAS	MLM	59	4	Wu et al. (2015)
	Rapeseed	419	19,167	GWAS	GLM & MLM	98	3	Chen et al. (2018)
	Rice	312	183,884	GWAS	MLM	14	2	Zhao et al. (2018)
	Maize	269	43,737	GWAS & QTL mapping	GLM & MLM	5	3	Zhao et al. (2018)

Pop: population; GWAS: genomewide association study; SSA: selective sweep analysis; GP: genomic prediction; QTL: quantitative trait loci; CIM: composite interval mapping; MLM: mixed linear model; LM; linear model; GLM: general linear model; MRA: multiple regression association; EMMAX: efficient mixed-model association expedited; MLMM: multi-locus mixed model; mrMLM: multi-locus random-SNP-effect mixed linear model of root traits, breeding efforts to date have focused on altering aboveground traits (Diederichsen et al. 2006; Heller and Byczyńska 2015). Root traits are essential for water availability as well as nutrient uptakes, making them important for crop improvement under drought conditions (Narayanan et al. 2014). Flax cultivars with deeper and denser root systems could access water more efficiently from deeper soil layers, especially in rain-fed agricultural systems (Dash et al. 2014; Sertse et al. 2019; Soto-Cerda et al. 2019). Flax cultivars with improved drought tolerance are crucial for producing plants with high fiber yields with minimum adverse impact on fiber quality (El-Hariri et al. 2005). Therefore, the combined improvement of aboveground traits and root traits has the potential to produce well-adapted cultivars for fiber production. Due to the major threats posed by abiotic stresses, efforts are being made to enhance tolerance in flax. QTNs governing drought tolerance or root traits have been reported in flax (Sertse et al. 2019; Soto-Cerda et al. 2019, 2020). Root development is known to play a significant role in plant nutrient uptake, and consequently, both morphological and physiological traits are important for abiotic stress tolerance (Jia et al. 2019). Incorporating genetic information on the root architecture system into flax breeding practices would benefit efforts to enhance resource efficiency and/or stress tolerance. The recent development of image software tools and advances in HTP have enabled more refined studies of root architecture (Furbank and Tester 2011; Hartmann et al. 2011; Fahlgren et al. 2015). Applications of GWAS have also enabled the identification of important QTLs for root traits (Hochholdinger et al. 2018) and some have already been applied in wheat to develop elite genotypes (Wasson et al. 2014). In flax, a GWAS of 115 accessions grown hydroponically was phenotyped for 15 root and two shoot traits, as well as for the shoot to root dry weight ratio (Sertse et al. 2019). This study tested seven different models and identified 228 QTNs for 16 traits. Candidate genes at the QTN loci encoded GRAS transcription factors, mitogen-activated protein kinases, and auxin-related lateral organ boundary proteins. Plants rely on a wide range of protective and adaptive mechanisms against various abiotic stresses. A considerable number of GWAS studies have shown that individual root traits are important for breeding practices in crops such as cassava (Yonis et al. 2020), maize (Pace et al. 2015; Wang et al. 2019a), wheat (Li et al. 2019a), and barley (Jia et al. 2019). A GS analysis in cassava suggested that root-related traits could be predicted with higher accuracy than yield (Yonis et al. 2020). Indeed, a comprehensive knowledge of the root architecture system is crucial to understand its direct role in abiotic stress responses such as drought. Both GWAS and GS offer opportunities to expand our understanding of the roles of roots in abiotic stress tolerance and hold potential for indirect selection of the difficult-to-phenotype root system.

8.2.1.2 Drought Stress (DS) and Water Use Efficiency

In plant breeding, DS is considered one of the most significant abiotic stresses that negatively affects agricultural productivity worldwide (Shao et al. 2009). Drought and temperature fluctuations are major environmental threats that often lead to extensive socio-economic crises and limit agricultural crop productivity (Zhao and Dai 2015). In the year 2060, more than 50% of the earth's arable land's crop productivity will be limited by water scarcity (Dhankher and Foyer 2018). DS causes significant reduction not only in yield but also in harvest areas when it is combined with extreme temperature fluctuations (Lesk et al. 2016). For example, during 1961–2014, the estimated combined effect of drought and temperature stresses in maize, soybean, and wheat resulted in global yield losses of 11.6, 12.4, and 9.2%, respectively (Matiu et al. 2017). For effective plant growth, development, and productivity, water availability is crucial, and its deficit can result in lower yields or even plant death. For instance, flax fiber yield is highly dependent on water availability and can be reduced by as much as 35–50% when water deficits are experienced during the vegetative stage as a consequence of the high transpiration rate at this stage (Heller and Byczyńska 2015). So far in flax genetic improvement, few have achieved the development of drought resistant genotypes (Diederichsen et al. 2006; Qi et al. 2010; Sharma et al. 2012; Asgarinia et al. 2016). Recently, Serste et al. (2021) conducted a GWAS study for 11 different traits in irrigated (IR) and non-irrigated (NIR) fields for three years. Six of the 11 traits showed significant variations between IR and NIR conditions. Seven different GWAS models were used to identify QTNs associated with DS, and a total of 148 QTNs were found associated with at least one trait or stress index. Among these QTNs, 16 were deemed to have major effects because they accounted for more than 15% of the genetic variance. Genotypes such as CN101595, CN98566, and fiber types from China outperformed others under drought conditions and should be good drought-resistant resources in flax. Similarly, Soto-Cerda et al. (Soto-Cerda et al. 2020) investigated four agronomic and four root traits under DS and IR conditions using 170,534 SNPs from 418 diverse flax accessions. Two single- and three multi-locus models identified 118 QTNs for drought-related traits. Candidate genes were involved in drought-responsive pathways, root, and vascular tissue development. Similar types of GWAS studies for drought and waterlogging tolerance have also been performed in soybean (Liu et al. 2020), chrysanthemum (Su et al. 2019), maize (Zhang et al. 2013), and barley (Wehner et al. 2016).

Although the identification of QTLs for DS-related traits remains a challenge because of the polygenic nature of the traits, the magnitude of the genotype by environment interactions, and consequently the low heritability, the cited studies are promising. Enhanced research on flax is expected to lead to novel insights towards a better understanding of drought tolerance. Taken together, these studies should greatly facilitate marker-assisted breeding not only in flax but also in other species experiencing drought stress.

8.2.1.3 Heat Stress (HS)

Global average temperatures are predicted to increase by 1–4 °C by the end of the twenty-first century (Driedonks et al. 2016). This could threaten global crop production because these heightened temperatures may cause HS. The development of cultivars resistant to such abiotic stress is needed to ensure sustainability in agricultural production under unfavorable environmental conditions (Bita and Gerats 2013). Limited literature is available regarding the impact of HS on flax. One study reported that HS does not significantly affect flower production but contributes to a reduction in boll formation and seed set (Cross et al. 2003). To date, no GWAS has been conducted on HS-related traits in flax; this may be due to the lack of appropriate phenotyping methods for such complex traits and the inherent difficulties associated with controlling the HS conditions in field environments, thereby limiting measurements to the small number of individuals that could be handled in controlled environments. Reliable and accurate phenotyping is crucial for successful GWAS (Powell et al. 2012). This is why GWAS has been successfully applied to many more biotic and agronomic traits than to abiotic stress-related traits (Soto-Cerda et al. 2018; You et al. 2018; He et al. 2019). Recently, some studies have revealed the existence of QTLs associated with HS-related traits that are promising as molecular markers in breeding programs. GWAS was utilized to delve into heat tolerance in *Brassica napus* and identified key candidate genes associated with flowering, male sterility, pollen abortion, and others (Rahaman et al. 2018). Plants were exposed to different HS regimes during the flowering stage and examined for traits such as pollen sterility, sterile/aborted seeds in siliques, and number of siliques on the main raceme. Using 37,539 SNPs and 88 diverse accessions of *B. napus*, a total of 5, 8, and 7 QTNs were identified for the above traits, respectively (Rahaman et al. 2018). A number of the candidate genes proposed were tissue-specific and temporally expressed. A GWAS of sorghum was conducted to measure HS response for leaf firing (LF) and leaf blotching (LB) at the vegetative stage (Chen et al. 2017). These traits were assessed for up to three years at three locations. With 339 accessions and 13,987 SNPs, nine SNPs were highly associated with LF and five with LB. The candidate gene analysis showed that most of them were directly linked to known plant stress response genes including HS. Other similar GWAS as well as meta analyses also identified QTNs associated with HS tolerance and their underlying candidate genes (Acuña-Galindo et al. 2015; Lafarge et al. 2017; Maulana et al. 2018).

Refinement of the identified QTL would benefit candidate gene identification by narrowing down the search to more focused and relevant regions. Candidate genes can be used as transgenes to validate their functional role in HS tolerance. Several species have applied this strategy including *Arabidopsis* (Yokotani et al. 2008; Khurana et al. 2017), wheat (Zang et al. 2017), yeast, and rice (Qin et al. 2015).

8.2.1.4 Cold Stress (CS)

Cold or low temperature stresses are the most common type of environmental stresses to cause crop production losses in many climatic regions, whether temperate, tropical or subtropical (Sanghera et al. 2011). Prolonged CS disturbs various plant growth activities and causes growth retardation, late flowering, stem elongation, and other effects (Patel and Franklin 2009). For instance, losses of up to 26% were observed in rice exposed to a CS during the reproductive stage (Ye et al. 2009). CSs not only limit crop productivity but also determine crops' geographical distribution (Chinnusamy et al. 2007; Thakur et al. 2010). The intensity of the CSs can vary from chilling (0–15 °C) to freezing (sub-zero) (Sanghera et al. 2011).

Under CS, many plants, including grapevine, watermelon, *Arabidopsis*, and flax, can produce certain "counteracting" metabolites such as phenolics and flavonoids (Rivero et al. 2001; Król et al. 2015; Schulz et al. 2016; Tchoumtchoua et al. 2019). Flax is mostly grown in temperate regions and cultivars vary greatly in their ability to respond to CS. For instance, winter-type cultivars are generally more resistant to CS than spring types. The two most abundant phenolic compounds found in winter flax (methylated C-glycosylflavonoid swertisin and swertiajaponin) were not detected in spring flax (Tchoumtchoua et al. 2019).

A GWAS analysis performed on 150 accessions of rice landraces using 67,511 SNPs identified 26 SNPs significantly associated with cold tolerance, explaining from 26 to 33% of the phenotypic variation (Song et al. 2018). This study suggested that rice landraces are valuable sources of cold tolerance that could be capitalized upon in breeding. Several other rice GWAS identified between 47 and 67 QTLs each for CS-related traits (Wang et al. 2016a; Schläppi et al. 2017; Zhang et al. 2018). In maize, 275 QTLs were identified using 49,585 SNPs (Revilla et al. 2016). Interestingly, 47 flint inbreds harbored the favorable alleles for six major QTLs but only four dent type inbreds had the favorable alleles from the test crosses.

In summary, several genes have been reported in different species to be associated with CS, namely in *Oryza sativa* (Yoon et al. 2016), transgenic tobacco (Jin et al. 2016), *Verbena bonariensis* (Wang et al. 2020), *Arabidopsis thaliana* (Dai et al. 2007; Visconti et al. 2019), transgenic cotton (Hao et al. 2018), *B. napus* (Savitch et al. 2005), *Zoyia japonica* (Kim et al. 2020), and *Hordeum vulgare* (Gierczik et al. 2019). The identification of functional polymorphism(s) in these genes remains a daunting task. However, further validation of these genes via orthologous gene identification and GWAS must be conducted for CS-related traits in order to apply them with confidence in flax breeding.

8.2.1.5 Salt Stress (SS)

SS is another abiotic stress that causes significant reductions in crop productivity worldwide (Munns and Tester 2008). The annual global losses due to soil salinization in irrigated areas have been estimated to be upward of 27B US$ (Qadir et al. 2014). One study estimated that nearly half of the global irrigated land area is affected by soil salinity, and about one-fifth of the arable lands (Qiao et al. 2014). SS negatively affects crop growth, indirectly by affecting soil water potential and directly by affecting water uptake. The soil water potential decreases with an increase in ion concentration, thereby effectively reducing water availability to the plant (Medina et al. 2020). Generally, SS affects plant's growth in comparable ways to DS and response to both stresses shares physiological mechanisms such as the closure of stomata, the loss of turgescence and photosynthetic rate, the development of reactive oxygen species

(ROS), an increase of heat-shock proteins (HSPs), and others (Zhu 2000; Chaves et al. 2008; Rosyara et al. 2016).

Soil salinity is a growing problem in agriculture production worldwide. Several strategies have been employed to understand flax's response to SS but these were met with limited success; they include gene expression analysis, biochemical markers, and biotechnological applications (Mchughen and Swartz 1984; Mchughen 1987; El-Beltagi et al. 2008; Hashem et al. 2011; Yu et al. 2014). To identify SS responsive QTLs in crops, several GWAS have been conducted in rice (Cui et al. 2018; Lekklar et al. 2019; Liu et al. 2019a), barley (Mwando et al. 2020), soybean (Do et al. 2019), *Arabidopsis* (Deolu-Ajayi et al. 2019), and alfalfa (Liu et al. 2019f; Medina et al. 2020). One study evaluated seed-germination-related traits of 350 diverse accessions of barley grown under control and SS conditions (Mwando et al. 2020). Approximately 24 K markers were used to identify 19 loci containing 52 significant markers that were associated with salt tolerance. A combined GWAS and GS approach was used in alfalfa (Medina et al. 2020). Using three phenotypic data sets and several GWAS models, 27 SNPs were associated with salt tolerance. Both barley and alfalfa studies illustrate the potential for GWAS and GS, not only to identify QTLs for salt tolerance, but also in practical breeding applications.

Several candidate genes have been hypothesized to play a role in salt tolerance in plants including rice (Tang et al. 2019), *Arabidopsis* (Gao et al. 2003; Zhang et al. 2019), soybean (Nguyen et al. 2019), wheat (Jiang et al. 2014), potato (Wang et al. 2019b), and rapeseed (Yang et al. 2019). These candidate genes may be useful in marker-assisted breeding for developing salt resilient genotypes. More insights into the molecular mechanisms underlying SS response is required because they remain generally obscure and, this knowledge is nearly completely lacking in flax. Likewise, the identification by GWAS of genes or loci involved in salt tolerance will provide additional avenues to address this growing problem.

8.2.1.6 Cadmium Stress (Cd)

Cd is a non-essential toxic metal that is widespread in water, and in the atmosphere and is found in both plants and animals. Cd in soils may come from various sources such as industrial emission, the extensive and prolonged use of fertilizers, atmospheric deposition, and public waste (Tanhuanpää et al. 2007; Ismael et al. 2019). Kidney and bone damage-causing itai-itai disease in Japan has made consumers and scientists aware of the negative effects of Cd when consumed by humans (Roberts 2014). Soils vary in Cd content due to both natural and anthropogenic processes, with phosphate fertilizers being one of the main culprit sources of Cd. Cd is toxic to most of the plant cells, even at low leaf concentrations of 5–10 µg/g (White and Brown 2010), although few species have adapted to Cd toxicity and can tolerate as much as 100 µg/g (Broadley et al. 2001; Verbruggen et al. 2009; Lux et al. 2010). Flax seeds bio-accumulate Cd ranging from 0.232–0.716 ppm (Booker 2019). Among crops grown in Canada, flax and durum wheat are particularly sensitive to soil Cd stress (Jiao et al. 2004). Cd is not evenly distributed in the plant and its partitioning is temporally modulated throughout its life cycle. In flax for example, Cd accumulates in the following tissues in decreasing order: root > stem > leaf > seed (Angelova et al. 2004). Cd accumulation is critical in tissues that are consumed because it is toxic to both plants and animals, including humans (Godt et al. 2006; Clemens et al. 2013). Some of the toxic effects include liver and renal damages, and bone demineralization (Satarug and Moore 2004). Cd uptake by roots can be translocated to the aboveground organs, including seeds, which can have toxic effects on the consumer (Li et al. 2017). GWAS analyses of Cd accumulation have been performed in several species including rapeseed (Chen et al. 2018), bread wheat (Hussain et al. 2020), rice (Zhao et al. 2018), maize (Zhao et al. 2018), and barley (Wu et al. 2015). Several genes have also been reported to be involved in Cd tolerance, such as *OsMTP1* (Yuan et al. 2012; Das et al. 2016), *OsABCG36*, *AtABCC1*, *AtABCC2* (Park et al. 2012; Fu et al. 2019), *AtHMA3*, *AtHMA4* (Verret et al. 2004; Morel et al. 2009), and *GmWRKY142* (Cai et al. 2020). To date, little is known about flax's response at the molecular level to Cd exposure. The flax orthologues to some of the above Cd-associated genes have been identified in a genomewide analysis and are suggested as candidate genes (Khan et al. 2020). Following validation, these genes can eventually accelerate flax breeding improvement towards the development of low Cd varieties.

Recently, the Cd accumulation in 418 diverse accessions of *B. napus* was evaluated by GWAS with a total of 19,167 SNPs (Chen et al. 2018). Twenty-five QTLs identified with 98 SNPs and dispersed on 15 chromosomes described 3.49–7.57% of the variation associated with root Cd concentration. In this study, 32 Cd-related genes were explored in regions of 0.33–497.97 kb from the QTNs. In maize, 269 accessions were grown in contaminated soil to identify loci controlling Cd accumulation at the seedling and maturity stages (Zhao et al. 2018). The GWAS identified the major QTL qLCd2 which explained 39.8% of the average phenotypic variance across experiments. The candidate gene analyses from the rapeseed and maize studies included genes encoding a cadmium-sensitivity protein, a cadmium/zinc-transporting ATPase, and an iron-regulated transporter.

Taken together, such findings will facilitate future studies and could be useful for flax breeding. Cd content could become an important trade barrier due to its toxicity and its presence in food and feed is undesirable. In addition, Cd is also toxic to plants and affects their growth. Thus, developing varieties with low Cd accumulation is desirable because it is the most economical and environmentally-friendly way to solve this critical issue.

8.2.2 Identification of Gene Families Associated with Abiotic Stresses

Genome-wide investigation of the gene families associated with abiotic and other types of stresses mainly relies on protein–protein sequences from model species such as *Arabidopsis,* and/or hidden Markov model (HMM) searches (Khan et al. 2018a, 2018b; Khan et al. 2020). Generally this approach is combined with gene expression studies and qRT-PCR analyses to verify the functionality of the orthologous genes (Wu et al. 2017; Khan et al. 2018b). Advanced resources in both genomics and bioinformatics have facilitated the study of the evolution of plant gene families. The knowledge developed in one plant species can be leveraged to identify abiotic stress-related gene families across multiple species based on sequence homology and/or synteny. Plant genes belonging to families are often clustered. Characterization of an individual family member is paramount to understanding their functional diversity. Both sequence homology and synteny could lead to the identification of orthologous candidate genes in the species of interest. In the last decade, a number of studies have shown that transcription factors including AP2/ERF (Xie et al. 2019), MYB (Dubos et al. 2010), NAC (Liu et al. 2019e), WRKY (Jiang et al. 2017), bZIP (Dröge-Laser et al. 2018), homeobox (Khan et al. 2018b), and bHLH (Sun et al. 2018) play a vital role in abiotic stress tolerance. Functional genomic studies in many plant species have also validated candidate genes associated with abiotic stress. Several examples can be cited such as an improved root architecture system and enhanced SS activity in rice (*RCc3*) (Li et al. 2018b), drought and SS tolerance (*OsMYB6)* (Tang et al. 2019), heat tolerance (*TaMBF1c*) (Qin et al. 2015), low temperature tolerance (*CBF1, CBF2,* or *CBF3*) leading to increased freezing tolerance (Gilmour et al. 2004), and low Cd accumulation (*OsHMA3* and *OsABCG36*) (Sasaki et al. 2014; Fu et al. 2019). Using such strategy, we recently identified nine candidate genes for Cd accumulation in flax (Khan et al. 2020). These genes hold potential to assist in solving the Cd toxicity problem in flax. As a whole, these functional genomics studies have greatly contributed to the identification of genes involved in abiotic stress and the underlying mechanisms by which plants respond to abiotic stresses.

GWAS and genome-wide gene family analyses are complementary. Studies have shown that bioinformatics and functional investigation of candidate genes associated with abiotic stress were synergistic (Shaban et al. 2018; Agarwal et al. 2019; Yan et al. 2019; Zhang et al. 2020b). Genome-wide gene family studies usually provide insights into evolutionary events such as gene duplication rate of evolution, and an understanding of the phylogenetic relationships (Wu et al. 2017). However, it is unable to provide a direct association with a phenotypic trait such as is accomplished by GWAS. In the last decade, the genomic resources available in public databases have facilitated the evolutionary studies of gene families. As such, publicly available genomic information has significantly empowered comparative gene family analyses across species to reveal their functional diversity. The outcome will provide focus to further the functional genomic characterization of these genes towards the improvement of stress tolerance in crops. A list of gene families which mediate response to abiotic stress tolerance in several species is provided (Table 8.2). Finding and mining candidate genes that are involved in abiotic stress response is an important step to unraveling and manipulating stress tolerance in flax.

Table 8.2 List of abiotic stress-related gene families and their functions

Abiotic stress-related gene families	Functions	References
Lipoxygenase gene family	Biotic and abiotic stresses	Shaban et al. (2018)
Basic leucine zipper (bZIP) transcription factor	Heat, salinity, and drought	Agarwal et al. (2019)
WRKY transcription factors	Multiple abiotic stresses	Yan et al. (2019)
Mitogen-activated protein kinase kinase kinases (MAPKKKs)	Drought stress	Zhang et al. (2020b)
Superoxide dismutase (SOD)	Drought, heat, cold and salinity	Verma et al. (2019)
Apetala2/Ethylene responsive factor (AP2/ERF) transcription factor	Hormone and abiotic stresses	Xie et al. (2019)
MYB transcription factors	Biotic and abiotic stresses	Dubos et al. (2010)
NAC transcription factor	Abiotic stress	Liu et al. (2019e)
Basic helix-loop-helix protein (bHLH) transcription factor	Drought, salt, and cold stress	Sun et al. (2018)
Sugars will eventually be exported transporters (SWEET)	Biotic and abiotic stresses	Miao et al. (2017)
Heat shock proteins (HSPs)	Biotic and abiotic stresses	Schöffl et al. (1998)
Phospholipase D Gene	Abiotic stress	Lu et al. (2019)
SQUAMOSA promoter binding protein (SBP)-box gene family	Abiotic stress	Zhang et al. (2020a)
Late embryogenesis abundant (LEA)	Abiotic stress	Liu et al. (2019c)
Protein phosphatases (PP2C)	Abiotic stress	Khan et al. (2019)
ATP-binding cassette (ABC)	Biotic and abiotic stresses	Khan et al. (2020)
Heavy metal ATPase (HMA)	Metals toxicity	Li et al. (2015)
Cation diffusion facilitator (CDF)	Metal ion uptake and transport in plants	Li et al. (2018c)
Plant metal tolerance proteins (MTPs)	Metals toxicity	Liu et al. (2019d)

8.2.3 *Novel Breeding Strategies Using Genomic Selection (GS) and Genetic Simulation*

GS and genetic simulation have recently emerged as promising and effective breeding strategies. GS is designed to generate genetic estimated breeding values (GEBVs) for many traits with the objectives of high prediction accuracy, low cost and reduced breeding cycles. Similarly, both GS and genetic simulation hold potential in improving the effectiveness in cross prediction and selection of breeding parents. In flax breeding programs, crosses are made to generate segregating populations with favorable genetic variation; however, prediction of cross performance and selection of parents remain challenging. The application of GS and genetic simulation can help address some of these challenges due to the remarkable increase in the amount of high quality genomic sequence data and the decline in the cost of high-throughput sequencing of the last several years (Bevan et al. 2017). Here, we will outline the potential of their applications in flax breeding programs.

8.2.3.1 Genomic Selection (GS)

GS is a type of marker-assisted selection (MAS) that is based either on highly dense random genomewide markers or on the QTLs identified from GWAS associated with the target trait(s). The main purpose of GS is to improve selection efficiency, precision, and decision-making by predicting GEBVs in breeding programs. GS takes advantage of a dense marker saturation or QTLs to predict phenotypes solely based on genotypic information. The initial concept of GS was introduced in 2001 (Meuwissen et al. 2001). Since then, remarkable progress has been made with this approach for improvement of biotic, abiotic and agronomic associated traits in species such as flax (Wang et al. 2019a; Lan et al. 2020), wheat (Bassi et al. 2016), maize (Albrecht et al. 2011), and soybean (Matei et al. 2018) to name a few. For abiotic stress-related traits, GS has been applied to drought (Shikha et al. 2017; Li et al. 2018a; Velazco et al. 2019; Wang et al. 2019c), salt (Medina et al. 2020), heat (Yuan et al. 2018), and cold stresses (Jähne et al. 2019).

GS success hinges on two main elements: statistical models and the choice of markers. Many GS models have been developed and tested for multiple traits with inconsistent success rates as evaluated based on their accuracy and the genetic gains made from selection. For example, seven different GS models were tested for agronomic traits in maize (Shikha et al. 2017). Of those, Bayes B had a higher prediction accuracy than random regression (RR), least absolute shrinkage and selection operator (LASSO), Elastic net (EN), Bayes A, random forest (RF), and reproducing kernel hilbert space (RKHS). Several additional studies investigating the potential for GS models to predict abiotic stress-related traits including DS, SS, HS, and CS are summarized (Table 8.3). The overall accuracy and performance of the GS models depend on several factors such as the model performance, the complexity and heritability of the traits, the density of markers, the total number of SNPs, and marker selection (Desta and Ortiz 2014). At the moment, the ridge regression best linear unbiased prediction (RR-BLUP) model is commonly used to predict abiotic stress-related traits and it often outperformed most other models (Table 8.3). However, it is quite possible that other models would prove superior if the choice of a different subset of markers or other experimental designs had been used. For instance, the machine learning methods such as support vector machine (SVM) and RF outperformed RR-BLUP for yield in alfalfa (Medina et al. 2020).

Selection of markers has been proven to be a critical factor for improving prediction accuracy of GEBVs. For instance, GS models based on QTLs as fixed effects consistently outperformed random SNP selection in flax (Wang et al. 2019a; Lan et al. 2020). These QTLs were identified using several single- and multi-locus GWAS

Table 8.3 Models commonly used for genomic selection to predict abiotic stress-related traits in crops

Models tested	Best model	Trait	Species	References
RR, LASSO, EN, Bayes A, Bayes B, RF, RKHS	Bayes B	DS	Maize	Shikha et al. (2017)
RR-BLUP	RR-BLUP	DS	Maize	Wang et al. (2019c)
GBLUP	GBLUP	DS	Maize	Dias et al. (2018)
RR-BLUP	RR-BLUP	DS & HS	Maize	Yuan et al. (2018)
RKHS, GBLUP	RKHS	DS	Rice	Bhandari et al. (2019)
RR-BLUP, BRR, LASSO	RR-BLUP	DS	Chickpea	Li et al. (2018a)
GBLUP	GBLUP	DS	Sorghum	Velazco et al. (2019)
RR-BLUP	RR-BLUP	CS	Soybean	Jähne et al. (2019)
RR-BLUP, Bayes A, Bayes B, Bayes C, BRR, LASSO, SVM, RF	SVM & RF	SS	Alfalfa	Medina et al. (2020)

RR: Ridge Regression; LASSO: Least Absolute Shrinkage and Selection Operator; EN: Elastic Net, RKHS: Reproducing Kernel Hilbert Space; RF: Random Forest; RR-BLUP: Ridge-Regression Best Linear Unbiased Prediction; BRR: Bayesian Ridge Regression; GBLUP: Genomic Best Linear Unbiased Prediction; SVM: Support Vector Machine; DS: Drought stress; HS: Heat stress; CS: Cold stress; SS: Salt stress

statistical methods. The GS results using these QTLs also demonstrate the robustness and reliability of the QTLs identified. The high accuracy obtained with the QTLs is hypothesized to be a consequence of the reduction in background noises through the removal of unrelated markers. This noise may arise from genomewide unrelated markers and through imputation (Rutkoski et al. 2012). Generally, using QTLs is more beneficial for improving GS accuracy than using genomewide random SNPs. (Deshmukh et al. 2014). The combined use of single- and multi-locus GWAS models for QTL identification that serves as marker input for testing GS models is recommended to achieve high accuracy and consistency in GEBVs (Lan et al. 2020) (Fig. 8.1).

GWAS in flax have already identified a large number of QTNs associated with root characteristics and drought tolerance traits (Sertse et al. 2019, 2021; Soto-Cerda et al. 2019, 2020). However, no GS studies have been reported for these traits to date. With the current increased frequency and intensity of occurrence of these stresses, the popularity of these methods is predicted to grow in response to the need to create crops that are better able to withstand these stresses. The GS approach is anticipated to facilitate future abiotic stress studies in flax specifically for drought, Cd toxicity, and other important traits.

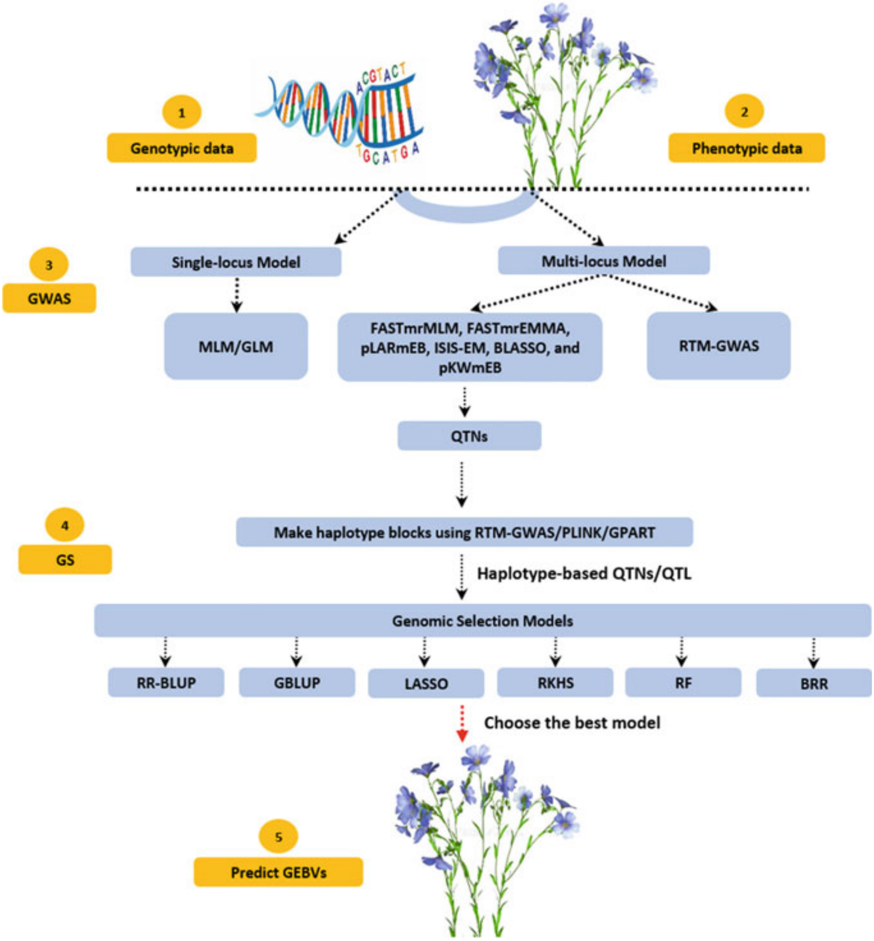

Fig. 8.1 A comprehensive approach for genomic selection (GS) based on GWAS-derived QTL as markers for flax breeding

8.2.3.2 Strategies for Cross Prediction and Parent Selection

The fundamental objective of a breeding program is to develop superior cultivars for specific target traits under a wide range of environmental conditions. Traditional breeding is time consuming and largely relies on the breeder's experience and plant phenotypes, often producing undesirable results such as inaccurate predictions and low efficiency. Cross-breeding using single crosses, double crosses or backcrosses, followed by offspring selection such as pedigree selection and single-seed descent, is the most used method in plant breeding. Parent evaluation and selection are the first step of cross-breeding. To broaden the narrow genetic base of Canadian flax cultivars (You et al. 2016), a core collection of 407 accessions has been compiled from the world flax collections (Diederichsen et al. 2013; Soto-Cerda et al. 2013). These accessions have been phenotypically evaluated in multiple environments for several years and at multiple locations (You et al. 2017). Although breeders can select genotypes with superior phenotypes as parents for crossing, the accuracy and efficiency of parent selection are impeded by unknown genetic structures and allelic make-up of the potential parents. Making crosses using all accessions of the core collection is impractical due to the extensive resources that would be required. The limited number of crosses possible narrows the probability to recover the best recombinants. Genetic simulation can enrich the cross-breeding strategy through effective cross prediction, best parent selection and the generation of hundreds to thousands of virtual crosses in a short time. Genetic simulation is applied to breeding whereby genomic data of parents are used to predict the traits of interest and then virtual crosses and their progenies are generated using computer tools. The use of computer simulation tools can assist breeding schemes and allow the exploration of a wide range of hypotheses in a limited time for complex traits at a low cost. Recently, with the development of extensive genomic datasets and through the use of computer simulations, high accuracy in virtual cross-breeding in a limited time have been made possible. These advances promise to greatly facilitate selection of suitable breeding schemes, allowing enhanced prediction in the selection for complex traits, and increasing efficiency in cross-breeding. As genome sequencing grows to be more cost-effective, genetic simulation becomes a useful approach to save time and solve the problems that cannot easily be solved via conventional breeding approaches.

In cross-breeding, parents are crossed to generate segregating populations from which a superior inbred progeny is selected. Many crosses are made and the value of a particular cross depends on the performance of its best progeny and its mean progeny performance (population mean). A critical issue in a breeding program is the need to evaluate these crosses cost-effectively and efficiently. Thus, the important elements to consider in using genetic simulation include (i) phenotyping and genotyping of potential parents as a training population for the development of GS models and simulation of a progeny population for each cross based on genomic data of parents, and (ii) the application of the developed GS models to predict GEBVs of all progenies of a cross. The cross performance is mainly evaluated according to usefulness criteria that is a function of progeny population mean and genetic variance (Zhong and Jannink 2007) (Fig. 8.2). This strategy will ensure the best progeny crosses, the most effective breeding methods, and high breeding accuracy and efficiency in flax breeding. Such strategy has been used to predict crosses in crops such as wheat (Lado et al. 2017; Yao et al. 2018), and maize (Bernardo 2015) as well as in theoretical studies (Zhong and Jannink 2007).

Software Tools and Their Applications in Flax Breeding Simulation

Numerous tools are available to simulate breeding schemes. Some of the recent tools for breeding scheme simulation are listed in Table 8.4. The pSBVB is a simulation tool for complex phenotypic traits and to compute the genomic matrix relationships

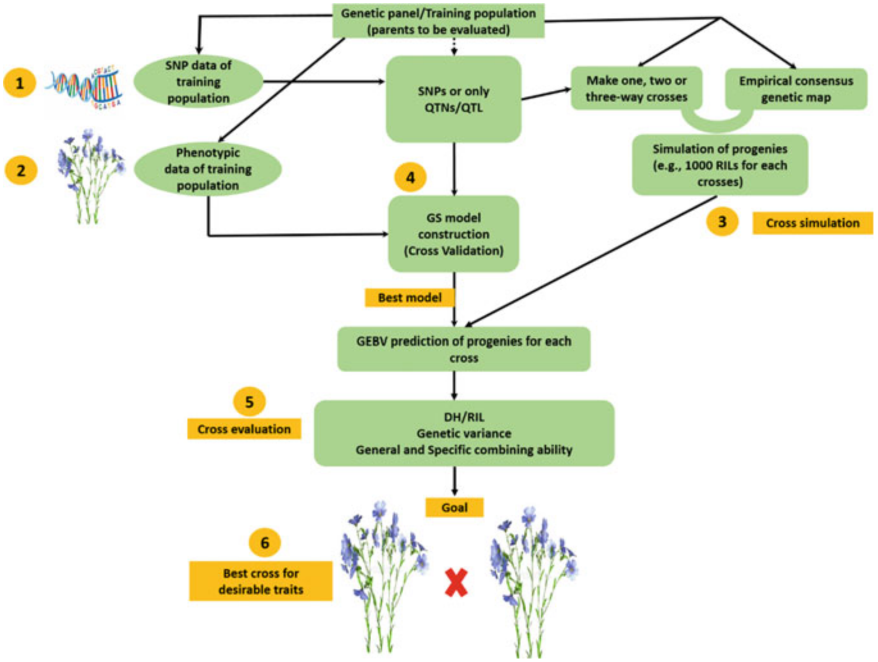

Fig. 8.2 A strategy of cross prediction and parent selection using genomic selection and genetic simulation

among polyploids (Zingaretti et al. 2019). PedigreeSim is used for both diploid and tetraploid species and predicts pedigrees and cross progeny (Voorrips and Maliepaard 2012). Similarly, the ADAM-plant and QuLinePlus models can predict breeding outcomes of cross- and self-pollinated crops (Hoyos-Villegas et al. 2019; Liu et al. 2019b). The ADAM-plant simulation works for predicting self-pollinated crop plants (Liu et al. 2019b) and it also has the ability to trace significant genetic changes in a set of populations under various scenarios. Simulation of progeny populations requires recombination rates between all adjacent markers, and MareyMap is a useful tool to estimate them through a consensus genetic map as a training data set (Siberchicot et al. 2017).

Cross Prediction and Parent Evaluation in Flax Breeding

Recently, we performed cross prediction and parent evaluation through GS and genetic simulation for five traits including seed yield (YLD), days to maturity (DTM), oil content (OIL), linolenic acid content (LIN), and powdery mildew resistance (PM) in flax (unpublished data). A total of 290 flax linseed accessions were evaluated, generating 4096 (290 × 289 /2) possible virtual single crosses with a partial diallel cross scheme. Ten GS models were evaluated, and the RR-BLUP model displayed

Table 8.4 Main features of different phenotypic-genotypic simulation models

Model	Feature	References
pSBVB	Simulate any number of complex phenotypes	Zingaretti et al. (2019)
PedigreeSim	Simulate both diploid and tetraploid species and predict pedigrees and cross populations	Voorrips and Maliepaard (2012)
PhenotypeSimulator	Predict multiple traits with multiple underlying genetic loci	Meyer and Birney (2018)
ADAM-plant	A tool that models breeding schemes for both self- and cross-pollinated crop plants	Liu et al. (2019b)
QuLinePlus	A simulation model for cross-pollinated crops	Hoyos-Villegas et al. (2019)
MareyMap	A tool to calculate recombination rates of all markers on a physical map based on a training genetic map	Siberchicot et al. (2017)
AlphaSim	Enable the simulation of multiple aspects of breeding programs such as haplotype sequences and pedigrees, perform selection and simulate new generations	Faux et al. (2016)
Phenosim	Simulate phenotypes for testing in GWAS	Günther et al. (2011)
SLiM	A simulation framework that enables modeling of a wide variety of complex evolutionary scenarios	Haller and Messer (2019)
G2P	A simulation tool for both genotype and phenotype	Tang and Liu (2019)
SimPed	A simulation tool to generate haplotype and genotype data for pedigree structures	Leal et al. (2005)

a higher prediction ability than the other models. General combining ability (GCA) and specific combining ability (SCA) were calculated for the simulated doubled haploid (DH) and recombinant inbred line (RIL) populations and a significant linear relationship between GCA and SCA ($R^2 = 0.93$–0.98) was obtained for all five traits (Fig. 8.3), indicating that a parent of higher GCA is more likely to generate high performance crosses with other parents. Also, a high correlation was observed between the mid-parent GEBVs and SCA ($R^2 = 0.93$–0.98) as shown in Fig. 8.4, suggesting that high mid-parent GEBVs of crosses are a good indicator of the potential of crosses. The results of this study provide a solid foundation for future cross-breeding studies.

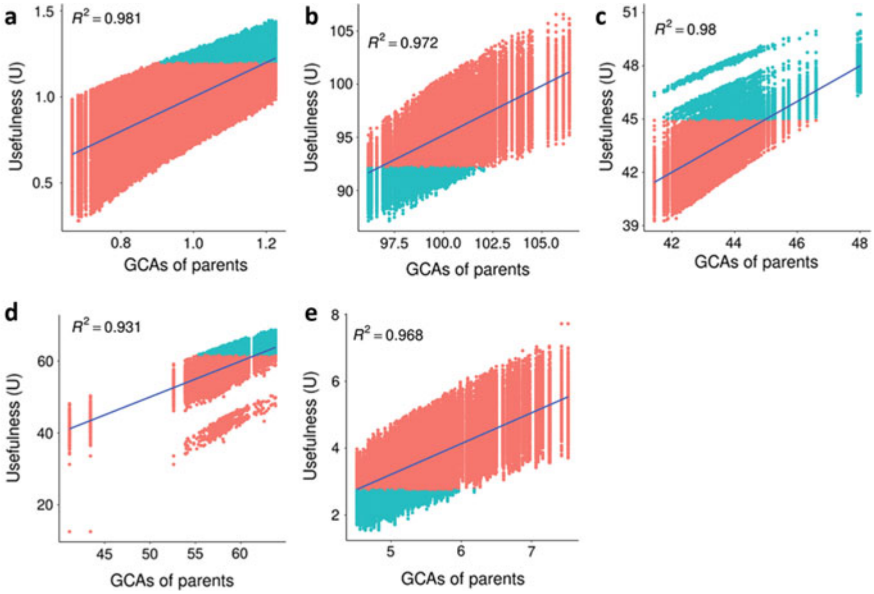

Fig. 8.3 Relationship between GCAs of parents and usefulness (U) of crosses for five traits. The blue dots represent the top 10% of crosses based on U of crosses at a 10% selection rate. **a** Seed yield (t/ha); **b** Days to maturity (days); **c** Oil content (%); **d** Linolenic acid content (%); **e** Powdery mildew resistance in flax. *Source* unpublished data

8.3 Future Perspectives

The significant advancement in GS and genetic simulation offers new opportunities to further improve abiotic trait-marker associations, cross-breeding accuracy, and breeding selection efficiency. Genomic studies investigating the relationships between root traits and drought tolerance have led to the identification of 521 QTLs in flax (Sertse et al. 2019, 2021; Soto-Cerda et al. 2019, 2020). The data generated in these studies can be capitalized upon in GS and genetic simulation to gain further insights into the genetic complexities of these traits. For future flax genetic improvement, it is also vital to expand the portfolio of abiotic stress-related traits to include Cd stress for example. To date, cross-breeding and parent selection remain a major challenge in plant breeding. The GS and genetic simulation combined strategy offers opportunities to improve the accuracy and efficiency of cross-breeding in flax by predicting the best crosses. However, the use of genetic simulation in flax remains in its infancy and much awaits to be done. Further investigations on the identification of significant QTLs, the use of HTP, and accuracy improvement of GS and genetic simulation for both biotic and abiotic traits in flax are warranted.

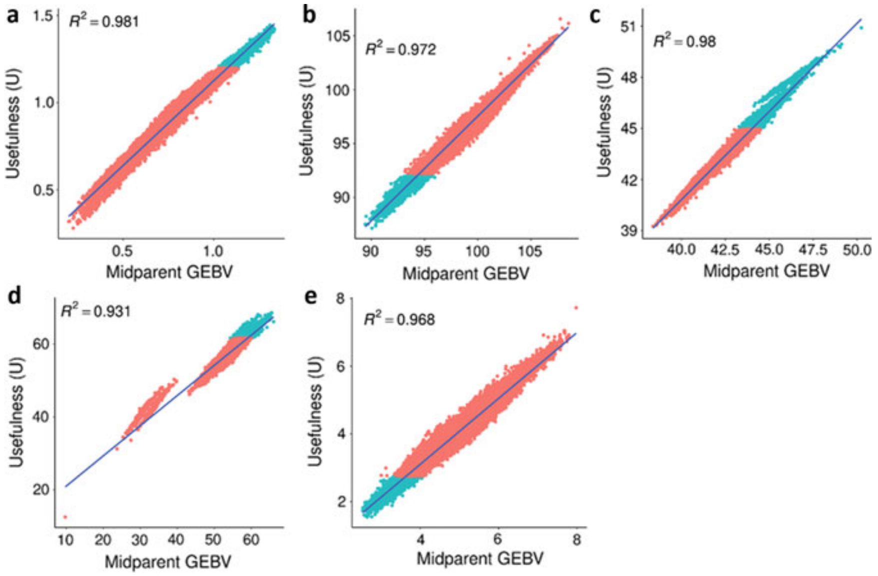

Fig. 8.4 Relationship between the mid-parent GEBV values and usefulness (U) of single crosses for five traits. The blue dots represent the top 10% of crosses based on U of crosses at a 10% selection rate. **a** Seed yield (t/ha); **b** Days to maturity (days); **c** Oil content (%); **d** Linolenic acid content (%); **e** Powdery mildew resistance in flax. *Source* unpublished data

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 55:477–492

Agarwal P, Baranwal VK, Khurana P (2019) Genome-wide analysis of bZIP transcription factors in wheat and functional characterization of a *TabZIP* under abiotic stress. Sci Rep 9:4608

Albrecht T, Wimmer V, Auinger H-J, Erbe M, Knaak C et al (2011) Genome-based prediction of testcross values in maize. Theor Appl Genet 123:339

Altshuler D, Daly MJ, Lander ES (2008) Genetic mapping in human disease. Science 322:881–888

Amarasinghe SL, Su S, Dong X, Zappia L, Ritchie ME et al (2020) Opportunities and challenges in long-read sequencing data analysis. Genome Biol 21:30

Angelova V, Ivanova R, Delibaltova V, Ivanov K (2004) Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp). Ind Crops Prod 19:197–205

Asgarinia P, Mirlohi A, Saeidi G, Mohamadi Mirik AA, Gheysari M et al (2016) Selection criteria for assessing drought tolerance in a segregating population of flax (*Linum usitatissimum* L.). Can J Plant Sci 97:424–437

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216

Bailey-Serres J, Parker JE, Ainsworth EA, Oldroyd GED, Schroeder JI (2019) Genetic strategies for improving crop yields. Nature 575:109–118

Bassi FM, Bentley AR, Charmet G, Ortiz R, Crossa J (2016) Breeding schemes for the implementation of genomic selection in wheat (*Triticum* spp.). Plant Sci 242:23–36

Bernardo R (2015) Genomewide selection of parental inbreds: classes of loci and virtual biparental populations. Crop Sci 55:2586–2595

Bevan MW, Uauy C, Wulff BBH, Zhou J, Krasileva K et al (2017) Genomic innovation for crop improvement. Nature 543:346–354

Bhandari A, Bartholomé J, Cao-Hamadoun T-V, Kumari N, Frouin J et al (2019) Selection of trait-specific markers and multi-environment models improve genomic predictive ability in rice. PLoS One 14:e0208871

Bita C, 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

Booker HM (2019) Flax breeding and cultivar registration in canada. In: Cullis CA (ed) Genetics and genomics of linum. Springer International Publishing, Cham, Switzerland, pp 39–62

Broadley MR, Willey NJ, Wilkins JC, Baker AJM, Mead A et al (2001) Phylogenetic variation in heavy metal accumulation in angiosperms. New Phytol 152:9–27

Brown C, Alexander P, Arneth A, Holman I, Rounsevell M (2019) Achievement of Paris climate goals unlikely due to time lags in the land system. Nat Clim Chang 9:203–208

Cai Z, Xian P, Wang H, Lin R, Lian T et al (2020) Transcription factor *GmWRKY142* confers cadmium resistance by up-regulating the cadmium tolerance 1-like genes. Front Plant Sci 11:724

Chaves MM, Flexas J, Pinheiro C (2008) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551–560

Chen J, Chopra R, Hayes C, Morris G, Marla S et al (2017) Genome-wide association study of developing leaves' heat tolerance during vegetative growth stages in a sorghum association panel. Plant Genome 10. https://doi.org/10.3835/plantgenome2016.3809.0091

Chen L, Wan H, Qian J, Guo J, Sun C et al (2018) Genome-wide association study of cadmium accumulation at the seedling stage in rapeseed (*Brassica napus* L.). Front Plant Sci 9:375

Chinnusamy V, Zhu J, Zhu JK (2007) Cold stress regulation of gene expression in plants. Trends Plant Sci 12:444–451

Ciesla A, Mitula F, Misztal L, Fedorowicz-Stronska O, Janicka S et al (2016) A role for barley calcium-dependent protein kinase CPK2a in the response to drought. Front Plant Sci 7:1550

Clemens S, Aarts MG, Thomine S, Verbruggen N (2013) Plant science: the key to preventing slow cadmium poisoning. Trends Plant Sci 18:92–99

Cloutier S, Ragupathy R, Miranda E, Radovanovic N, Reimer E et al (2012) Integrated consensus genetic and physical maps of flax (*Linum usitatissimum* L.). Theor Appl Genet 125:1783–1795

Cross RH, Mckay SAB, Mchughen AG, Bonham-Smith PC (2003) Heat-stress effects on reproduction and seed set in *Linum usitatissimum* L. (flax). Plant Cell Environ 26:1013–1020

Cui Y, Zhang F, Zhou Y (2018) The application of multi-locus GWAS for the detection of salt-tolerance loci in rice. Front Plant Sci 9:1464

Dai X, Xu Y, Ma Q, Xu W, Wang T et al (2007) Overexpression of an *R1R2R3* MYB gene, *OsMYB3R*, increases tolerance to freezing, drought, and salt stress in transgenic *Arabidopsis*. Plant Physiol 143:1739–1751

Das N, Bhattacharya S, Maiti MK (2016) Enhanced cadmium accumulation and tolerance in transgenic tobacco overexpressing rice metal tolerance protein gene *OsMTP1* is promising for phytoremediation. Plant Physiol Biochem 105:297–309

Dash PK, Cao Y, Jailani AK, Gupta P, Venglat P et al (2014) Genome-wide analysis of drought induced gene expression changes in flax (*Linum usitatissimum*). GM Crops Food 5:106–119

Deolu-Ajayi AO, Meyer AJ, Haring MA, Julkowska MM, Testerink C (2019) Genetic loci associated with early salt stress responses of roots. iScience 21:458–473

Deshmukh R, Sonah H, Patil G, Chen W, Prince S et al (2014) Integrating omic approaches for abiotic stress tolerance in soybean. Front Plant Sci 5:244

Desta ZA, Ortiz R (2014) Genomic selection: genome-wide prediction in plant improvement. Trends Plant Sci 19:592–601

Dhankher OP, Foyer CH (2018) Climate resilient crops for improving global food security and safety. Plant Cell Environ 41:877–884

Dias KODG, Gezan SA, Guimarães CT, Nazarian A, da Costa e Silva L et al (2018) Improving accuracies of genomic predictions for drought tolerance in maize by joint modeling of additive and dominance effects in multi-environment trials. Heredity 121:24–37

Diederichsen RT, Zhuchenko AA, Richards KW (2006) Screening for broad adaptation in 96 flax (*Linum usitatissimum* L.) accessions under dry and warm conditions in Canada and Russia. Plant Genet Resour Newsl 146:9–16

Diederichsen A, Kusters PM, Kessler D, Bainas Z, Gugel RK (2013) Assembling a core collection from the flax world collection maintained by plant gene resources of Canada. Genet Resour Crop Evol 60:1479–1485

Do TD, Vuong TD, Dunn D, Clubb M, Valliyodan B et al (2019) Identification of new loci for salt tolerance in soybean by high-resolution genome-wide association mapping. BMC Genomics 20:318

Driedonks N, Rieu I, Vriezen WH (2016) Breeding for plant heat tolerance at vegetative and reproductive stages. Plant Reprod 29:67–79

Dröge-Laser W, Snoek BL, Snel B, Weiste C (2018) The *Arabidopsis* bZIP transcription factor family—an update. Curr Opin Plant Biol 45:36–49

Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C et al (2010) MYB transcription factors in *Arabidopsis*. Trends Plant Sci 15:573–581

El-Beltagi HS, Salama ZA, El Hariri DM (2008) Some biochemical markers for evaluation of flax cultivars under salt stress conditions. J Nat Fibers 5:316–330

El-Hariri DM, Al-Kordy MA, Hassanein MS, Ahmed MA (2005) Partition of photosynthates and energy production in different flax cultivars. J Nat Fibers 1:1–15

Fahlgren N, Gehan MA, Baxter I (2015) Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. Curr Opin Plant Biol 24:93–99

Faux AM, Gorjanc G, Gaynor RC, Battagin M, Edwards SM et al (2016) AlphaSim: software for breeding program simulation. Plant Genome 9. https://doi.org/10.3835/plantgenome2016.3802.0013

Feng ZT, Deng YQ, Fan H, Sun QJ, Sui N et al (2014) Effects of NaCl stress on the growth and photosynthetic characteristics of *Ulmus pumila* L. seedlings in sand culture. Photosynthetica 52:313–320

Flowers TJC, TD (2008) Salinity tolerance in halophytes. New Phytol 179:945–963

Fu YB, Yang MH, Zeng F, Biligetu B (2017) Searching for an accurate marker-based prediction of an individual quantitative trait in molecular plant breeding. Front Plant Sci 8:1182

Fu S, Lu Y, Zhang X, Yang G, Chao D et al (2019) The ABC transporter *ABCG36* is required for cadmium tolerance in rice. J Exp Bot 70:5909–5918

Furbank RT, Tester M (2011) Phenomics—technologies to relieve the phenotyping bottleneck. Trends Plant Sci 16:635–644

Gao X, Ren Z, Zhao Y, Zhang H (2003) Overexpression of *SOD2* increases salt tolerance of *Arabidopsis*. Plant Physiol 133:1873–1881

Gierczik K, Székely A, Ahres M, Marozsán-Tóth Z, Vashegyi I et al (2019) Overexpression of two upstream phospholipid signaling genes improves cold stress response and hypoxia tolerance, but leads to developmental abnormalities in barley. Plant Mol Biol Rep 37:314–326

Gilmour SJ, Fowler SG, Thomashow MF (2004) *Arabidopsis* transcriptional activators *CBF1, CBF2*, and *CBF3* have matching functional activities. Plant Mol Biol 54:767–781

Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P et al (2006) The toxicity of cadmium and resulting hazards for human health. J Occup Med Toxicol 1:22

Günther T, Gawenda I, Schmid KJ (2011) phenosim—a software to simulate phenotypes for testing in genome-wide association studies. BMC Bioinform 12:265

Guo J, Li Y, Han G, Song J, Wang B (2018) NaCl markedly improved the reproductive capacity of the euhalophyte *Suaeda salsa*. Funct Plant Biol 45:350–361

Hall LM, Booker H, Siloto RMP, Jhala AJ, Weselake RJ (2016) Flax (*Linum usitatissimum* L.). In: McKeon TA, Hayes DG, Hildebrand DF, Weselake RJ (eds) Industrial oil crops. AOCS Press, pp 157–194

Haller BC, Messer PW (2019) SLiM 3: forward genetic simulations beyond the wright–fisher model. Mol Biol Evol 36:632–637

Hao Y-Q, Lu G-Q, Wang L-H, Wang C-L, Guo H-M et al (2018) Overexpression of *AmDUF1517* enhanced tolerance to salinity, drought, and cold stress in transgenic cotton. J Integr Agric 17:2204–2214

Hartmann A, Czauderna T, Hoffmann R, Stein N, Schreiber F (2011) HTPheno: an image analysis pipeline for high-throughput plant phenotyping. BMC Bioinform 12:148

Hashem HA, Bassuony FM, Hassanein RA, Baraka DM, Khalil RR (2011) Stigmasterol seed treatment alleviates the drastic effect of NaCL and improves quality and yield in flax plants. Aust J Crop Sci 5:1858–1867

Hassan TU, Bano A, Naz I (2017) Alleviation of heavy metals toxicity by the application of plant growth promoting rhizobacteria and effects on wheat grown in saline sodic field. Int J Phytoremediation 19:522–529

Hawkesford MJ, Griffiths S (2019) Exploiting genetic variation in nitrogen use efficiency for cereal crop improvement. Curr Opin Plant Biol 49:35–42

He L, Xiao J, Rashid KY, Yao Z, Li P et al (2019) Genome-wide association studies for pasmo resistance in flax (*Linum usitatissimum* L.). Front Plant Sci 9:1982

Heller K, Byczyńska M (2015) The impact of environmental factors and applied agronomy on quantitative and qualitative traits of flax fiber. J Nat Fibers 12:26–38

Hirschhorn JN (2009) Genomewide association studies–illuminating biologic pathways. N Engl J Med 360:1699–1701

Hochholdinger F, Yu P, Marcon C (2018) Genetic control of root system development in maize. Trends Plant Sci 23:79–88

Hocking PJ, Kirkegaard JA, Angus JF, Gibson AH, Koetz EA (1997) Comparison of canola, Indian mustard and Linola in two contrasting environments. I. Effects of nitrogen fertilizer on dry-matter production, seed yield and seed quality. Field Crops Res 49:107–125

Hopkin M (2005) Climate change: world round-up. Nature

Hoyos-Villegas V, Arief VN, Yang W-H, Sun M, DeLacy IH et al (2019) QuLinePlus: extending plant breeding strategy and genetic model simulation to cross-pollinated populations—case studies in forage breeding. Heredity 122:684–695

Hussain W, Campbell MT, Jarquin D, Walia H, Morota G (2020) Variance heterogeneity genome-wide mapping for cadmium in bread wheat reveals novel genomic loci and epistatic interactions. Plant Genome 13. https://doi.org/10.1002/tpg1002.20011

Ismael MA, Elyamine AM, Moussa MG, Cai M, Zhao X et al (2019) Cadmium in plants: uptake, toxicity, and its interactions with selenium fertilizers. Metallomics 11:255–277

Jähne F, Balko C, Hahn V, Würschum T, Leiser WL (2019) Cold stress tolerance of soybeans during flowering: QTL mapping and efficient selection strategies under controlled conditions. Plant Breed 138:708–720

Jia Z, Liu Y, Gruber BD, Neumann K, Kilian B et al (2019) Genetic dissection of root system architectural traits in spring barley. Front Plant Sci 10:400

Jiang Q, Hu Z, Zhang H, Ma Y (2014) Overexpression of *GmDREB1* improves salt tolerance in transgenic wheat and leaf protein response to high salinity. Crop J 2:120–131

Jiang J, Ma S, Ye N, Jiang M, Cao J et al (2017) WRKY transcription factors in plant responses to stresses. J Integr Plant Biol 59:86–101

Jiao Y, Grant CA, Bailey LD (2004) Effects of phosphorus and zinc fertilizer on cadmium uptake and distribution in flax and durum wheat. J Sci Food Agric 84:777–785

Jin C, Huang X-S, Li K-Q, Yin H, Li L-T et al (2016) Overexpression of a bHLH1 transcription factor of *Pyrus ussuriensis* confers enhanced cold tolerance and increases expression of stress-responsive genes. Front Plant Sci 7:441

Kar G, Kumar A, Martha M (2007) Water use efficiency and crop coefficients of dry season oilseed crops. Agric Water Manage 87:73–82

Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD et al (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468:647–652

Khadivi-Khub A (2014) Regression association analysis of fruit traits with molecular markers in cherries. Plant Syst Evol 300:1163–1173

Khan N, Hu C-m, Khan WA, Wang W, Ke H et al (2018b) Genome-wide identification, classification, and expression pattern of homeobox gene family in *Brassica rapa* under various stresses. Sci Rep 8:16265

Khan N, Hu C-m, Amjad Khan W, Hou X (2018a) Genome-wide identification, classification, and expression divergence of glutathione-transferase family in *Brassica rapa* under multiple hormone treatments. Biomed Res Int 2018:6023457

Khan N, Ke H, Hu C-M, Naseri E, Haider MS et al (2019) Genome-wide identification, evolution, and transcriptional profiling of *PP2C* gene family in *Brassica rapa.* Biomed Res Int 2019:2965035–2965035

Khan N, You FM, Datla R, Ravichandran S, Jia B, et al. (2020) Genome-wide identification of ATP binding cassette (ABC) transporter and heavy metal associated (HMA) gene families in flax (*Linum usitatissimum* L.). BMC Genomics 21:722

Khurana N, Sharma N, Khurana P (2017) Overexpression of a heat stress inducible, wheat myo-inositol-1-phosphate synthase 2 (*TaMIPS2*) confers tolerance to various abiotic stresses in *Arabidopsis thaliana.* Agri Gene 6:24–30

Kim Y-J, Yang D-H, Park M-Y, Sun H-J, Song P-S et al (2020) Overexpression of *Zoysia ZjCIGR1* gene confers cold stress resistance to zoysiagrass. Plant Biotechnol Rep 14:21–31

Król A, Amarowicz R, Weidner S (2015) The effects of cold stress on the phenolic compounds and antioxidant capacity of grapevine (*Vitis vinifera* L.) leaves. J Plant Physiol 189:97–104

Lado B, Battenfield S, Guzmán C, Quincke M, Singh RP et al (2017) Strategies for selecting crosses using genomic prediction in two wheat breeding programs. Plant Genome 10. https://doi.org/10.3835/plantgenome2016.3812.0128

Lafarge T, Bueno C, Frouin J, Jacquin L, Courtois B, et al. (2017) Genome-wide association analysis for heat tolerance at flowering detected a large set of genes involved in adaptation to thermal and other stresses. PLoS One 12:e0171254

Lan S, Zheng C, Hauck K, McCausland M, Duguid SD et al (2020) Genomic prediction accuracy of seven breeding selection traits improved by QTL identification in flax. Int J Mol Sci 21:1577

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

Leal SM, Yan K, Müller-Myhsok B (2005) SimPed: a simulation program to generate haplotype and genotype data for pedigree structures. Hum Hered 60:119–122

Lekklar C, Pongpanich M, Suriya-arunroj D, Chinpongpanich A, Tsai H et al (2019) Genome-wide association study for salinity tolerance at the flowering stage in a panel of rice accessions from Thailand. BMC Genomics 20:76

Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. Nature 529:84–87

Li D, Xu X, Hu X, Liu Q, Wang Z et al (2015) Genome-wide analysis and heavy metal-induced expression profiling of the *HMA* gene family in *Populus trichocarpa.* Front Plant Sci 6:1149

Li K, Yu H, Li T, Chen G, Huang F (2017) Cadmium accumulation characteristics of low-cadmium rice (*Oryza sativa* L.) line and F(1) hybrids grown in cadmium-contaminated soils. Environ Sci Pollut Res Int 24:17566–17576

Li X, Chen R, Chu Y, Huang J, Jin L et al (2018b) Overexpression of RCc3 improves root system architecture and enhances salt tolerance in rice. Plant Physiol Biochem 130:566–576

Li X, Wu Y, Li B, He W, Yang Y et al (2018c) Genome-wide identification and expression analysis of the cation diffusion facilitator gene family in turnip under diverse metal ion stresses. Front Genet 9:103

Li H, Rasheed A, Hickey LT, He Z (2018a) Fast-forwarding genetic gain. Trends Plant Sci 23:184–186

Li Y, Guan K, Schnitkey GD, DeLucia E, Peng B (2019b) Excessive rainfall leads to maize yield loss of a comparable magnitude to extreme drought in the United States. Glob Chang Biol 25:2325–2337

Li T, Ma J, Zou Y, Chen G, Ding P et al (2019a) Quantitative trait loci for seeding root traits and the relationships between root and agronomic traits in common wheat. Genome 63:27–36

Liu J, Gao Y, Tang Y, Wang D, Chen X et al (2019d) Genome-wide identification, comprehensive gene feature, evolution, and expression analysis of plant metal tolerance proteins in tobacco under heavy metal toxicity. Front Genet 10:345

Liu M, Ma Z, Sun W, Huang L, Wu Q et al (2019e) Genome-wide analysis of the NAC transcription factor family in Tartary buckwheat (*Fagopyrum tataricum*). BMC Genomics 20:113

Liu H, Xing M, Yang W, Mu X, Wang X et al (2019c) Genome-wide identification of and functional insights into the late embryogenesis abundant (LEA) gene family in bread wheat (*Triticum aestivum*). Sci Rep 9:13375

Liu C, Chen K, Zhao X, Wang X, Shen C et al (2019a) Identification of genes for salt tolerance and yield-related traits in rice plants grown hydroponically and under saline field conditions by genome-wide association study. Rice 12:88

Liu H, Tessema BB, Jensen J, Cericola F, Andersen JR et al (2019b) ADAM-Plant: a software for stochastic simulations of plant breeding from molecular to phenotypic level and from simple selection to complex speed breeding programs. Front Plant Sci 9:1926

Liu Z, Li H, Gou Z, Zhang Y, Wang X et al (2020) Genome-wide association study of soybean seed germination under drought stress. Mol Genet Genom 295:661–673

Liu X-P, Hawkins C, Peel MD, Yu L-X (2019f) Genetic loci associated with salt tolerance in advanced breeding populations of tetraploid alfalfa using genome-wide association studies. Plant Genome 12. https://doi.org/10.3835/plantgenome2018.3805.0026

Lu S, Fadlalla T, Tang S, Li L, Ali U et al (2019) Genome-wide analysis of phospholipase d gene family and profiling of phospholipids under abiotic stresses in *Brassica napus*. Plant Cell Physiol 60:1556–1566

Lux A, Martinka M, Vaculík M, White PJ (2010) Root responses to cadmium in the rhizosphere: a review. J Exp Bot 62:21–37

Mardis ER (2008) The impact of next-generation sequencing technology on genetics. Trends Genet 24:133–141

Matei G, Woyann LG, Milioli AS, De Bem OI, Zdziarski AD et al (2018) Genomic selection in soybean: accuracy and time gain in relation to phenotypic selection. Mol Breeding 38:117

Mathew I, Shimelis H, Shayanowako AIT, Laing M, Chaplot V (2019) Genome-wide association study of drought tolerance and biomass allocation in wheat. PLoS One 14:e0225383

Matiu M, Ankerst DP, Menzel A (2017) Interactions between temperature and drought in global and regional crop yield variability during 1961–2014. PLoS One 12:e0178339

Maulana F, Ayalew H, Anderson JD, Kumssa TT, Huang W et al (2018) Genome-wide association mapping of seedling heat tolerance in winter wheat. Front Plant Sci 9:1272

Mchughen A (1987) Salt tolerance through increased vigor in a flax line (STS-II) selected for salt tolerance in vitro. Theor Appl Genet 74:727–732

Mchughen A, Swartz M (1984) A tissue-culture derived salt-tolerant line of flax (*Linum usitatissimum*). J Plant Physiol 117:109–117

Medina CA, Hawkins C, Liu X-P, Peel M, Yu L-X (2020) Genome-wide association and prediction of traits related to salt tolerance in autotetraploid alfalfa (*Medicago sativa* L.). Int J Mol Sci 21:3361

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

Meyer HV, Birney E (2018) PhenotypeSimulator: a comprehensive framework for simulating multi-trait, multi-locus genotype to phenotype relationships. Bioinformatics 34:2951–2956

Miao H, Sun P, Liu Q, Miao Y, Liu J et al (2017) Genome-wide analyses of SWEET family proteins reveal involvement in fruit development and abiotic/biotic stress responses in banana. Sci Rep 7:3536

Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N et al (2009) *AtHMA3*, a P_{1B}-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*. Plant Physiol 149:894–904

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Mwando E, Han Y, Angessa TT, Zhou G, Hill CB et al (2020) Genome-wide association study of salinity tolerance during germination in barley (*Hordeum vulgare* L.). Front Plant Sci 11:118

Narayanan S, Mohan A, Gill KS, Prasad PV (2014) Variability of root traits in spring wheat germplasm. PLoS One 9:e100317

Naveed SA, Zhang F, Zhang J, Zheng T-Q, Meng L-J et al (2018) Identification of QTN and candidate genes for salinity tolerance at the germination and seedling stages in rice by genome-wide association analyses. Sci Rep 8:6505

Nguyen QH, Vu LTK, Nguyen LTN, Pham NTT, Nguyen YTH et al (2019) Overexpression of the *GmDREB6* gene enhances proline accumulation and salt tolerance in genetically modified soybean plants. Sci Rep 9:19663

Nicod J, Davies R, Cai N et al (2016) Genome-wide association of multiple complex traits in outbred mice by ultra-low-coverage sequencing. Nat Genet 48:912–918

Pace J, Gardner C, Romay C, Ganapathysubramanian B, Lübberstedt T (2015) Genome-wide association analysis of seedling root development in maize (*Zea mays* L.). BMC Genomics 16:47–47

Panuccio MR, Sorgonà A, Rizzo M, Cacco G (2009) Cadmium adsorption on vermiculite, zeolite and pumice: batch experimental studies. J Environ Manage 90:364–374

Park J, Song WY, Ko D, Eom Y, Hansen TH et al (2012) The phytochelatin transporters *AtABCC1* and *AtABCC2* mediate tolerance to cadmium and mercury. Plant J 69:278–288

Patel D, Franklin KA (2009) Temperature-regulation of plant architecture. Plant Signal Behav 4:577–579

Pierart A, Shahid M, Séjalon-Delmas N, Dumat C (2015) Antimony bioavailability: knowledge and research perspectives for sustainable agricultures. J Hazard Mater 289:219–234

Powell N, Ji X, Ravash R, Edlington J, Dolferus R (2012) Yield stability for cereals in a changing climate. Funct Plant Biol 39:539–552

Qadir M, Quillérou E, Nangia V, Murtaza G, Singh M et al (2014) Economics of salt-induced land degradation and restoration. Nat Resour Forum 38:282–295

Qi X, Wang X, Xu J, Zhang J, Mi J (2010) Drought-resistance evaluation of flax germplasm at adult plant stage. Sci Agric Sin 43:3076–3087

Qiao G, Zhang X, Jiang J, Liu M, Han X et al (2014) Comparative proteomic analysis of responses to salt stress in chinese willow (*Salix matsudana* Koidz). Plant Mol Biol Rep 32:814–827

Qin D, Wang F, Geng X, Zhang L, Yao Y et al (2015) Overexpression of heat stress-responsive *TaMBF1c*, a wheat (*Triticum aestivum* L.) multiprotein bridging factor, confers heat tolerance in both yeast and rice. Plant Mol Biol 87:31–45

Rahaman M, Mamidi S, Rahman M (2018) Genome-wide association study of heat stress-tolerance traits in spring-type *Brassica napus* L. under controlled conditions. Crop J 6:115–125

Revilla P, Rodríguez VM, Ordás A, Rincent R, Charcosset A et al (2016) Association mapping for cold tolerance in two large maize inbred panels. BMC Plant Biol 16:127

Rivero RM, Ruiz JM, García PC, López-Lefebre LR, Sánchez E et al (2001) Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. Plant Sci 160:315–321

Roberts TL (2014) Cadmium and phosphorous fertilizers: the issues and the science. Procedia Eng 83:52–59

Rosyara UR, De Jong WS, Douches DS, Endelman JB (2016) Software for genome-wide association studies in autopolyploids and its application to potato. Plant Genome 9:1–10. https://doi.org/10.3835/plantgenome2015.3808.0073

Rutkoski J, Benson J, Jia Y, Brown-Guedira G, Jannink J-L et al (2012) Evaluation of genomic prediction methods for fusarium head blight resistance in wheat. Plant Genome 5. https://doi.org/10.3835/plantgenome2012.3802.0001

Salla V, Hardaway CJ, Sneddon J (2011) Preliminary investigation of *Spartina alterniflora* for phytoextraction of selected heavy metals in soils from Southwest Louisiana. Microchem J 97:207–212

Sanghera GS, Wani SH, Hussain W, Singh NB (2011) Engineering cold stress tolerance in crop plants. Curr Genomics 12:30–43

Sasaki A, Yamaji N, Ma JF (2014) Overexpression of *OsHMA3* enhances Cd tolerance and expression of Zn transporter genes in rice. J Exp Bot 65:6013–6021

Satarug S, Moore MR (2004) Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. Environ Health Perspect 112:1099–1103

Savitch LV, Allard G, Seki M, Robert LS, Tinker NA et al (2005) The effect of overexpression of two brassica CBF/DREB1-like transcription factors on photosynthetic capacity and freezing tolerance in *Brassica napus.* Plant Cell Physiol 46:1525–1539

Schläppi MR, Jackson AK, Eizenga GC, Wang A, Chu C et al (2017) Assessment of five chilling tolerance traits and GWAS mapping in rice using the USDA mini-core collection. Front Plant Sci 8:957

Schöffl F, Prändl R, Reindl A (1998) Regulation of the heat-shock response. Plant Physiol 117:1135–1141

Schulz E, Tohge T, Zuther E, Fernie AR, Hincha DK (2016) Flavonoids are determinants of freezing tolerance and cold acclimation in *Arabidopsis thaliana.* Sci Rep 6:34027

Sertse D, You FM, Ravichandran S, Cloutier S (2019) The complex genetic architecture of early root and shoot traits in flax revealed by genome-wide association analyses. Front Plant Sci 10:1483

Sertse D, You FM, Ravichandran S, et al (2021) Loci harboring genes with important role in drought and related abiotic stress responses in flax revealed by multiple GWAS models. Theor Appl Genet 134:191–212

Shaban M, Ahmed MM, Sun H, Ullah A, Zhu L (2018) Genome-wide identification of lipoxygenase gene family in cotton and functional characterization in response to abiotic stresses. BMC Genomics 19:599

Shahid M, Khalid S, Abbas G, Shahid N, Nadeem M et al (2015) Heavy metal stress and crop productivity. In: Hakeem KR (ed) Crop production and global environmental issues. Springer International Publishing, Cham, pp 1–25

Shao H-B, Chu L-Y, Jaleel CA, Manivannan P, Panneerselvam R et al (2009) Understanding water deficit stress-induced changes in the basic metabolism of higher plants—biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. Crit Rev Biotech 29:131–151

Sharma JCTS, Shivran RK, Chandra P (2012) Water requirement water use efficiency consumptive use yield and quality parameters of linseed (*Linum usitatissimum* L.) varieties as influenced by fertility levels irrigation scheduling. Adv Life Sci 1:180–182

Shikha M, Kanika A, Rao AR, Mallikarjuna MG, Gupta HS et al (2017) Genomic selection for drought tolerance using genome-wide SNPs in maize. Front Plant Sci 8:550

Siberchicot A, Bessy A, Guéguen L, Marais GA (2017) MareyMap online: a user-friendly web application and database service for estimating recombination rates using physical and genetic maps. Genome Biol Evol 9:2506–2509

Slama I, Abdelly C, Bouchereau A, Flowers T, Savouré A (2015) Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Ann Bot 115:433–447

Song J, Li J, Sun J, Hu T, Wu A et al (2018) Genome-wide association mapping for cold tolerance in a core collection of rice (*Oryza sativa* L.) landraces by using high-density single nucleotide polymorphism markers from specific-locus amplified fragment sequencing. Front Plant Sci 9:875

Soto-Cerda BJ, Cloutier S, Gajardo HA, Aravena G, Quian R (2019) Identifying drought-resilient flax genotypes and related-candidate genes based on stress indices, root traits and selective sweep. Euphytica 215:41

Soto-Cerda BJ, Cloutier S, Gajardo HA, Aravena G, Quian R et al (2020) Drought response of flax accessions and identification of quantitative trait nucleotides (QTNs) governing agronomic and root traits by genome-wide association analysis. Mol Breed 40:15

Soto-Cerda BJ, Diederichsen A, Ragupathy R, Cloutier S (2013) Genetic characterization of a core collection of flax (*Linum usitatissimum* L.) suitable for association mapping studies and evidence of divergent selection between fiber and linseed types. BMC Plant Biol 13:78

Soto-Cerda BJ, Cloutier S, Quian R, Gajardo HA, Olivos M et al (2018) Genome-wide association analysis of mucilage and hull content in flax (*Linum usitatissimum* L.) seeds. Int J Mol Sci 19

Su J, Zhang F, Chong X, Song A, Guan Z et al (2019) Genome-wide association study identifies favorable SNP alleles and candidate genes for waterlogging tolerance in chrysanthemums. Hort Res 6:21

Sukumaran S, Reynolds MP, Sansaloni C (2018) Genome-wide association analyses identify QTL hotspots for yield and component traits in durum wheat grown under yield potential, drought, and heat stress environments. Front Plant Sci 9:81

Sun X, Wang Y, Sui N (2018) Transcriptional regulation of bHLH during plant response to stress. Biochem Biophys Res Commun 503:397–401

Tam V, Patel N, Turcotte M, Bossé Y, Paré G et al (2019) Benefits and limitations of genome-wide association studies. Nat Rev Genet 20:467–484

Tang Y, Liu X (2019) G2P: a genome-wide-association-study simulation tool for genotype simulation, phenotype simulation and power evaluation. Bioinformatics 35:3852–3854

Tang Y, Bao X, Zhi Y, Wu Q, Guo Y et al (2019) Overexpression of a MYB family gene, *OsMYB6*, increases drought and salinity stress tolerance in transgenic rice. Front Plant Sci 10:168

Tanhuanpää P, Kalendar R, Schulman AH, Kiviharju E (2007) A major gene for grain cadmium accumulation in oat (*Avena sativa* L.). Genome 50:588–594

Tchoumtchoua J, Mathiron D, Pontarin N, Gagneul D, van Bohemen A-I et al (2019) Phenolic profiling of flax highlights contrasting patterns in winter and spring varieties. Molecules 24:4303

Thakur P, Kumar S, Malik JA, Berger JD, Nayyar H (2010) Cold stress effects on reproductive development in grain crops: an overview. Environ Exp 67:429–443

Velazco JG, Jordan DR, Mace ES, Hunt CH, Malosetti M et al (2019) Genomic prediction of grain yield and drought-adaptation capacity in sorghum is enhanced by multi-trait analysis. Front Plant Sci 10:997

Verbruggen N, Hermans C, Schat H (2009) Mechanisms to cope with arsenic or cadmium excess in plants. Curr Opin Plant Biol 12:364–372

Verma D, Lakhanpal N, Singh K (2019) Genome-wide identification and characterization of abiotic-stress responsive SOD (superoxide dismutase) gene family in *Brassica juncea* and *B. rapa.* BMC Genomics 20:227

Verret F, Gravot A, Auroy P, Leonhardt N, David P et al (2004) Overexpression of *AtHMA4* enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. FEBS Lett 576:306–312

Visconti S, D'Ambrosio C, Fiorillo A, Arena S, Muzi C, et al. (2019) Overexpression of 14-3-3 proteins enhances cold tolerance and increases levels of stress-responsive proteins of *Arabidopsis* plants. Plant Sci 289:110215

Visioni A, Tondelli A, Francia E, Pswarayi A, Malosetti M et al (2013) Genome-wide association mapping of frost tolerance in barley (*Hordeum vulgare* L.). BMC Genomics 14:424

Voorrips RE, Maliepaard CA (2012) The simulation of meiosis in diploid and tetraploid organisms using various genetic models. BMC Bioinform 13:248

Voss-Fels K, Snowdon RJ (2016) Understanding and utilizing crop genome diversity via high-resolution genotyping. Plant Biotech J 14:1086–1094

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9:244–252

Wang S-B, Feng J-Y, Ren W-L, Huang B, Zhou L et al (2016) Improving power and accuracy of genome-wide association studies via a multi-locus mixed linear model methodology. Sci Rep 6:19444

Wang D, Liu J, Li C, Kang H, Wang Y et al (2016) Genome-wide association mapping of cold tolerance genes at the seedling stage in rice. Rice 9:61

Wang X, Chen ZH, Yang C, Zhang X, Jin G et al (2018) Genomic adaptation to drought in wild barley is driven by edaphic natural selection at the tabigha evolution slope. Proc Natl Acad Sci USA 115:5223–5228

Wang L, Liu Y, Li D, Feng S, Yang J et al (2019b) Improving salt tolerance in potato through overexpression of *AtHKT1* gene. BMC Plant Biol 19:357

Wang H, Wei J, Li P, Wang Y, Ge Z et al (2019a) Integrating GWAS and gene expression analysis identifies candidate genes for root morphology traits in maize at the seedling stage. Genes (basel) 10:773

Wang N, Liu B, Liang X, Zhou Y, Song J et al (2019c) Genome-wide association study and genomic prediction analyses of drought stress tolerance in China in a collection of off-PVP maize inbred lines. Mol Breeding 39:113

Wang M-q, Huang Q-x, Lin P, Zeng Q-h, Li Y et al (2020) The overexpression of a transcription factor gene *VbWRKY32* enhances the cold tolerance in *Verbena bonariensis*. Front Plant Sci 10:1746

Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J 4:162–176

Wasson AP, Rebetzke GJ, Kirkegaard JA, Christopher J, Richards RA et al (2014) Soil coring at multiple field environments can directly quantify variation in deep root traits to select wheat genotypes for breeding. J Exp Bot 65:6231–6249

Wehner G, Balko C, Ordon F (2016) QTL for water use related traits in juvenile barley. Agron 6:62

White PJ, Brown PH (2010) Plant nutrition for sustainable development and global health. Ann Bot 105:1073–1080

Wu D, Sato K, Ma JF (2015) Genome-wide association mapping of cadmium accumulation in different organs of barley. New Phytol 208:817–829

Wu P, Wang W, Li Y, Hou X (2017) Divergent evolutionary patterns of the MAPK cascade genes in *Brassica rapa* and plant phylogenetics. Hort Res 4:17079

Xia H, Zheng X, Chen L, Gao H, Yang H et al (2014) Genetic differentiation revealed by selective loci of drought-responding EST-SSRs between upland and lowland rice in China. PLoS One 9:e106352

Xie Z, Nolan TM, Jiang H, Yin Y (2019) AP2/ERF transcription factor regulatory networks in hormone and abiotic stress responses in *Arabidopsis*. Front Plant Sci 10:228

Xiong T, Leveque T, Shahid M, Foucault Y, Mombo S et al (2014) Lead and cadmium phytoavailability and human bioaccessibility for vegetables exposed to soil or atmospheric pollution by process ultrafine particles. J Environ Qual 43:1593–1600

Yan H, Li M, Xiong Y, Wu J, Teixeira da Silva JA et al (2019) Genome-wide characterization, expression profile analysis of WRKY family genes in *Santalum album* and functional identification of their role in abiotic stress. Int J Mol Sci 20:5676

Yang H, Deng L, Liu H, Fan S, Hua W et al (2019) Overexpression of *BnaAOX1b* confers tolerance to osmotic and salt stress in rapeseed. G3-Genes Genom Genet 9:3501–3511

Yao J, Zhao D, Chen X, Zhang Y, Wang J (2018) Use of genomic selection and breeding simulation in cross prediction for improvement of yield and quality in wheat (*Triticum aestivum* L.). Crop J 6:353–365

Ye H, Du H, Tang N, Li X, Xiong L (2009) Identification and expression profiling analysis of TIFY family genes involved in stress and phytohormone responses in rice. Plant Mol Biol 71:291–305

Yokotani N, Ichikawa T, Kondou Y, Matsui M, Hirochika H et al (2008) Expression of rice heat stress transcription factor *OsHsfA2e* enhances tolerance to environmental stresses in transgenic *Arabidopsis*. Planta 227:957–967

Yonis BO, Pino Del Carpio D, Wolfe M, Jannink J-L, Kulakow P et al (2020) Improving root characterisation for genomic prediction in cassava. Sci Rep 10:8003

Yoon DH, Lee SS, Park HJ, Lyu JI, Chong WS et al (2016) Overexpression of *OsCYP19-4* increases tolerance to cold stress and enhances grain yield in rice (*Oryza sativa*). J Exp Bot 67:69–82

You FM, Duguid SD, Lam I, Cloutier S, Rashid KY et al (2016) Pedigrees and genetic base of flax cultivars registered in Canada. Can J Plant Sci 96:837–852

You FM, Xiao J, Li P, Yao Z, Jia G et al (2018) Chromosome-scale pseudomolecules refined by optical, physical and genetic maps in flax. Plant J 95:371–384

You FM, Jia G, Xiao J, Duguid SD, Rashid KY et al (2017) Genetic variability of 27 traits in a core collection of flax (*Linum usitatissimum* L.). Front Plant Sci 8

Yu Y, Huang W, Chen H, Wu G, Yuan H et al (2014) Identification of differentially expressed genes in flax (*Linum usitatissimum* L.) under saline–alkaline stress by digital gene expression. Gene 549:113–122

Yuan L, Yang S, Liu B, Zhang M, Wu K (2012) Molecular characterization of a rice metal tolerance protein, *OsMTP1*. Plant Cell Rep 31:67–79

Yuan Y, Cairns JE, Babu R, Gowda M, Makumbi D et al (2018) 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

Zang X, Geng X, Wang F, Liu Z, Zhang L et al (2017) Overexpression of wheat ferritin gene *TaFER-5B* enhances tolerance to heat stress and other abiotic stresses associated with the ROS scavenging. BMC Plant Biol 17:14

Zhang X, Tang B, Yu F, Li L, Wang M et al (2013) Identification of major QTL for waterlogging tolerance using genome-wide association and linkage mapping of maize seedlings. Plant Mol Biol Rep 31:594–606

Zhang M, Ye J, Xu Q, Feng Y, Yuan X et al (2018) Genome-wide association study of cold tolerance of Chinese *Indica* rice varieties at the bud burst stage. Plant Cell Rep 37:529–539

Zhang K, Cui H, Cao S, Yan L, Li M et al (2019) Overexpression of *CrCOMT* from *Carex rigescens* increases salt stress and modulates melatonin synthesis in *Arabidopsis thaliana*. Plant Cell Rep 38:1501–1514

Zhang D, Han Z, Li J, Qin H, Zhou L et al (2020) Genome-wide analysis of the SBP-box gene family transcription factors and their responses to abiotic stresses in tea (*Camellia sinensis*). Genomics 112:2194–2202

Zhang J-B, Wang X-P, Wang Y-C, Chen Y-H, Luo J-W et al (2020) Genome-wide identification and functional characterization of cotton (*Gossypium hirsutum*) *MAPKKK* gene family in response to drought stress. BMC Plant Biol 20:217

Zhao T, Dai A (2015) The magnitude and causes of global drought changes in the 21st century under a low-moderate emissions scenario. J Clim 28(11):4490–4512

Zhao K-F, Song J, Fan H, Zhou S, Zhao M (2010) Growth response to ionic and osmotic stress of NaCl in salt-tolerant and salt-sensitive maize. J Integr Plant Biol 52:468–475

Zhao J, Yang W, Zhang S, Yang T, Liu Q et al (2018) Genome-wide association study and candidate gene analysis of rice cadmium accumulation in grain in a diverse rice collection. Rice 11:61

Zhong S, Jannink J-L (2007) Using quantitative trait loci results to discriminate among crosses on the basis of their progeny mean and variance. Genetics 177:567–576

Zhou JC, Fu TT, Sui N, Guo JR, Feng G et al (2016) The role of salinity in seed maturation of the euhalophyte *Suaeda salsa*. Plant Biosyst 150:83–90

Zhu JK (2000) Genetic analysis of plant salt tolerance using *Arabidopsis*. Plant Physiol 124:941–948

Zhu JK (2016) Abiotic stress signaling and responses in plants. Cell 167:313–324

Zingaretti ML, Monfort A, Pérez-Enciso M (2019) pSBVB: a versatile simulation tool to evaluate genomic selection in polyploid species. G3-Genes Genom Genet 9:327–334