Chittaranjan Kole *Editor*

Genomic Designing for Abiotic Stress Resistant Technical Crops

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Dedicated to
Prof. R. B. Singh
Padma Bhushan Awardee, Former Chancellor, Central Agricultural University, Imphal, and
Past President, National Academy of Agricultural Sciences

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

Preface

Crop production is drastically affected due to external or environmental stresses. The biotic stresses cause significant yield losses in the range of 31–42% together with 6–20% loss during the post-harvest stage. The abiotic stresses also aggravate the situation with crop damage in the range of 6–20%. Understanding the mechanisms of interaction of plants with the biotic stresses caused by insects, bacteria, fungi, viruses, and 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 in 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 to 15% should be minimized. Therefore, increase in the crop yield as well as minimization of its loss should be practiced simultaneously focusing both on 'adaptation' and 'mitigation'.

Traditional plant breeding practiced in the last century contributed a lot to the science of crop genetic improvement. Classical plant breeding methods including selection, hybridization, polyploidy, and mutation effectively catered to the basic F^5 needs—food, feed, fiber, fuel, and furniture. The advent of molecular breeding and genetic engineering in the latter part of that 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 have facilitated identification of exactly the genes conferring resistance, tolerance or adaptability leading to gene discovery, allele mining and shuttle breeding which is turn opened up the scope for 'designing' or 'tailoring' crop genomes with resistance/tolerance to biotic and abiotic stresses.

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

The chapters of the 12 volumes of this twin book series entitled, "Genomic Designing for Biotic Stress Resistant Crops" and "Genomic Designing for Abiotic Stress Resistant Crops" will deliberate on different types of biotic and abiotic stresses and their effects on and interaction with crop plants; will enumerate the available genetic diversity with regard to biotic or abiotic stress resistance among cultivars; 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 Technical Crops" includes 11 chapters focused on Cassava, Coconut, Coffee, Cotton, Floricultural Crops, Jute, Mulberry, Rubber, Sugarcane, Tobacco, and Yam contributed by 73 scientists from 11 countries including Brazil, China, D. R. Congo, Finland, France, Ghana, India, Iran, Nigeria, Portugal, 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

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Abbreviations

^{13}C	Carbon isotope ratio
ABA	Abscisic acid
ABF3	Abscisic acid responsive elements-binding factor 3
ABRE	ABA responsive elements
ABS	Access-Benefit Sharing
ACC	1-aminocyclopropane-1-carboxylate oxygenase
ACT	Actin
ACT	Artemis Comparison Tool
ADH	Alcohol dehydrogenase
AE	ArrayExpress
AFLP	Amplified fragment length polymorphism
AhCMO	Atriplexhortensis choline monoxygenase
AI	Acid invertase
AICEM	All India Coordinated Experimental Trail of Mulberry
ALF	Abnormal leaf fall
ALMT	Aluminum-activated malate transporters
AM	Association mapping
AMT	Accurate mass and retention time tags
AMT	*Agrobacterium*-mediated transformation
AMT	Ammonium transporter
ANR	French Agency for Research
AP2	Apetala2
AP47	Clathrin-associated protein
Apt	Adeninephosphoribosyltransferase
APX	Ascorbate peroxidase
AQP	Aquaporin
ARF	Auxin response factor
ARR	Arabidopsis response regulator
ASFV	Average subordinate function value
ATAF	*Arabidopsis thaliana* activating factor
AtCBF3	*Arabidopsis thaliana* C-repeat binding factor 3

ATP	Adenosine triphosphate
AtSOS	*Arabidopsis thaliana* salt overly sensitive
a-TUB	Alpha tubulin
AVP1	*Arabidopsis thaliana* main H+-pyrophosphatase
AVROS	AlgemeneVerneiging Rubber Planters Oostkust Sumatra, Indonesia
BAC	Bacterial artificial chromosome
BBX	B-box
BBX19	B-box 19
BC	Backcross
BD	Bodjong Datar, Indonesia
BES	BRI1-EMS Suppressor
Bhle	Boeahygrometrica late embryogenesis
bHLH	Basic helix-loop-helix
BPM	BalaiPenelitian Perkebunan Medan, Indonesia
BSA-seq	Bulk segregant analysis-sequencing
bZIP	Basic leucine zipper
C2H2	Acetylene
Ca	Calcium
CaCc	*Coffea canephora* Subgenome in *Coffea arabica*
CaCe	*Coffea eugenioides* Subgenome in *Coffea arabica*
CAM	Crassulacean acid metabolism
CAMTA	Calmodulin binding transcription activator
CAPS	Cleaved amplified polymorphic sequence
Cas 9	CRISPR-associated protein 9
Cas	CRISPR-associated gene
CATAS	Chinese Academy of Tropical Agricultural Sciences
CATD	Catigan green dwarf
CBD	Convention on Biological Diversity
CBF	C-repeat/DRE binding factor 1
CBL	Casitas B-lineage lymphoma
cDNA	Complementary DNA
CEF	Forest Research Center
CgHSP70	Chrysanthemum heat shock protein
CGIAR	Consultative Group for International Agricultural Research
Ch	Chemara, Malaysia
Chl	Chlorophyll
CHM	C.H. Meares, Malaysia
CIM	Composite interval mapping
CIRAD	Centre de coopération internationale en recherche agronomique pour le
CIRAD	French Agricultural Research Centre for International Development
CK	Cytokinins
CKX1	Cytokinin oxidase/dehydrogenase
ClCBF1	*Chrysanthemum lavandulifolium* C-repeat/DRE binding factor 1
CLF	Coffee leaf rust

CmCIPK6	*Chrysanthemum morifolium* CBL-interacting protein kinase 6
CmNF	*Chrysanthemum morifolium* nuclear factor Y
CMS	Cytoplasmic male sterility
CmSHN3	*Chrysanthemum morifolium*cuticle biosynthesis
CNRA	Nickel and cobalt resistance protein
CNT	Carbon nanotube
CNV	Copy number variant
CodA	Choline oxidase
COG	Clusters of orthologous group
COR	Cold-regulated
COS	Conserved ortholog sequences
COSTREL	Combinatorial super transformation of transplastomic recipient lines
CPB	Cartagena Protocol on Biosafety
Cpt	Carnitinepalmitoyltransferase
CPT	CisPrenyltransferase
CRD	Completely randomized design
CRIJAF	Central Research Institute for Jute and Allied Fibres
CRISPR	Clustered regularly interspaced short palindromic repeats
CRT	Calreticulin
CSI	Chlorophyll stability index
CTD	Canopy temperature depression
CTRI	Central Tobacco Research Institute
CUC2	Cup-shaped cotyledon
cv	Cultivar
CWR	Crop wild relatives
CYCL	Cyclophilin
DArT	Diversity array technology
DAVID	Database for Annotation, Visualization, and Integrated Discovery
DB	Database
DcaHsf	*Dianthus caryophyllus* heat shock transcription factor
dCAPS	Derived CAPS
DDBJ	DNA Databank of Japan
ddRADseq	Double-digest restriction site-associated sequencing
DEG	Differentially expressed gene développement
DGE	Digital gene expression
DH	Doubled haploid
DHAR	Dehydro ascorbate reductase
DLY	Dry latex yield
DMAPP	Dimethylallyl pyrophosphate
DMRT	Duncan's multiple range test
Dof	DNA binding with one finger
DPPH	Diphenyl-1-picrylhydrazyl
DRAT	Departamento de Recursos Naturais, Ambiente e Território
DRE	DNA replication -related element

DREB	Dehydration responsive element binding
DREB1D	Dehydration-responsive element-binding protein 1D
DRF1	Dehydration-responsive factor 1
DRs	Disclosure requirements
DS	Drought-sensitive
dSm^{-1}	DeciSiemens per metre
dsRNA	Double-stranded RNA
DT	Drought-tolerant
DUS	Distinctiveness, uniformity, stability
DXS	Diffuse X-ray spectrometer
E2-UBQ2	Ubiquitin-conjugating enzyme
EaHSP70	*Erianthus arundinaceus* heat shock protein 70
EBI	European Bioinformatics Institute
EBP1	ErbB-3 Binding Protein 1
EC	Electrical conductivity
ECO_2	Elevated carbon dioxide
EF1	Elongation factor 1
EFO	European Patent Office
EFSA	European Food Safety Authority
Elf	Eukaryotic initiation factor
EMBL	European Molecular Biology Laboratory
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
EMS	Ethyl methane sulfonate
EPAMIG	Empresa de Pesquisa Agropecuária de Minas Gerais
ERF	Ethylene responsive factor
ERF	Ets-2 repressor factor
ESP	Exchangeable sodium percent
EST	Expressed sequence tag
ET	Elevated temperature
ETR	Electron transport rate
F1	Fillal 1
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FCT	Faculdade de Ciências e Tecnologia
FCTC	Framework Convention on Tobacco Control
FCV	Flue Cured Virginia
Fe	Iron
FFT	Fructan: fructan1-fructosyltransferase
FPP	Farnesyl pyrophosphate
Fro2	Ferric reduction oxidase 2
FS	Full sib
FT	Flowering locus
Fv/Fm	Chlorophyll fluorescence
GA	Gibberellic acid
GAB	Genomic-assisted breeding

GAM	Generation-wise assortative mating
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Gbp	Giga base pairs
GBS	Genotyping-by-sequencing
GC	Guanine-cytosine
GE	Genome editing
GE	Genetically editing
GE	Genetically engineered
GE	Genome engineering
GEA	Genomic expression archive
GEO	Gene expression omnibus
GEOBIOTEC	Unidade de Geobiociências, Geoengenharias e Geotecnologias
GGPP	Geranylgeranyldiphosphate
Gl	Glenshiel, Malaysia
GLDH	L-galactono-1,4-lactone dehydrogenase
GLN	Glutamine synthase
GLT	Glutamine oxoglutarate aminotransferase
GM	Genetically modified
GMD	GolmMetabolome Database
GMO	Genetically modified organism
GO	Gene ontology
GolS	Galactinol synthase
GPCRs	G-protein-coupled receptors
GPP	Geranyl pyrophosphate
GRF	Growth regulating factor
GRs	Genetic resources
gs	Stomatal conductance
GS	Genomic selection
Gs	Stomatal conductance
GSRs	Genome space sequence reads
GSS	Genomic survey sequences
GT	GondangTapen, Indonesia
GTP	Guanosine-5'-triphosphate
GUS	β-Glucuronidase
GWAS	Genome wide association study/studies
GYE	Grain yield efficiency
HAT	Hours after treatment
HbDHN	*Hevea brasiliensis* dehydrins
HD-ZIP	Homeodomain-leucine zipper
Hep2	Heparanase
HG	Homologous groups
Hil	Hilcroft, Sri Lanka
HMG-CoA	3-Hydroxy-3-methyl-glutaryl-coenzyme A reductase
HP	Promoter haplotypes
HR	Homology recombination

HRD	Hardy transcription factor
HRT	Hevea rubber transferase
HSF	Heat shock factor
Hsfs	Heat shock transcription factors
HSP	Heat shock protein
HTS	High-throughput sequencing
HVA1	*Hordeum vulgaris* abundant protein
IAA	Indole acetic acid
IAC	Instituto Agronômico de Campinas
IAN	Institute Agronomico du Norte, Brazil
IARI	Indian Agricultural Research Institute
IBSC	Institutional Biosafety Committee
ICAR	Indian Council of Agricultural Research
ICC	International coconut community
IDI	IsopentenyldiphosphateIsomerase
IgE	Immunoglobulin E
IGS	Intergenic spacer
IITA	International Institute of Tropical Agriculture
IM	Interval mapping
INCAPER	Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural
InDel-Seq	Insertion-deletion sequencing
INSDS	The International Nucleotide Sequence Database Collaboration
IPP	Isopentenyldiphosphate
IPR	Intellectual Property Rights
ipt	Isopentenyl transferase
IPT	Isopentenyltransferase
IPTG	Isopropyl β-D-1 thiogalactopyranoside
IRCA	Institute de Recherchessur le Caoutchouc, Ivory Coast
IRCI	Institute des Recherchessur le Caoutchouc, Indochina
IRD	Institute de la Recherche pour le Développement
IRRDB	International Rubber Research and Development Board
ISA	Instituto Superior de Agronomia
ISAAA	International Service for the Acquisition of Agri-biotech Applications
ISPS	Isoprene synthase
ISSR	Inter-simple sequence repeats
ISTR	Inverse sequence-tagged repeat
ITPGRFA	International Treaty for Plant Genetic Resources for Food and Agriculture
iTRAQ	Isobaric tags for relative and absolute quantitation
IUP	Isopentenol Utilization Pathway
KASP	Kompetitive allele-specific PCR
KASPar	KBiosciences competitive allelespecific PCR SNP genotyping system

KEGG	Kyoto Encyclopaedia of Genes and Genomes
KGGG	Kyoto Encyclopedia of Genes and Genomes
KRS	Kohong Rubber Estate, Thailand
LBD	Lateral Organ Boundaries Domain
LCBS'	Lands CaoutchoucBedrijven, Indonesia
LC-MS	Liquid chromatography-mass spectrometry
LD	Linkage disequilibrium
LD50	Lethal dose 50
LEA	Late embryogenesis abundant
LeNCED1	Tomato 9-cis-epoxycarotenoid dioxygenase
LFY	Transcription factor LEAFY
LIDAR	Light detection and ranging
LMOs	Living modified organisms
LTP	Lipid transfer protein
LTR	Long-terminal repeat
Lun	Lunderston, Malaysia
m5C	5-Methylcytosin
MAB	Marker-assisted breeding
MABC	Marker-assisted backcrossing
MACC	Marker-assisted complex or convergent crossing
MAGIC	Multi-parent advanced generation intercross
MALDI	Matrix-assisted laser desorption/ionization
MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight
MAP	Mitogen activated protein
MAPK	Mitogen-activated protein kinase
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MDF	Madre de Dios basin in Peru
MDH	Malate dehydrogenase
MegaN	Meganuclease
MEP	Methyl Erythritol 4-Phosphate
Mil	Milakande, Sri Lanka
miRNAs	MicroRNAs
MLT	Multi-locational trial
MMS	Methyl methane sulfonate
MN	Meganucleases
MoNA	Massbank of North America
MPa	Megapascals
MS	Mass spectrometry
MSNs	Mesoporous silica NPs
Mt1D	Mannitol-1-phosphate dehydrogenase
MtDREB	Medicago truncatulaDehydration responsive element binding
MVA	Mevalonic acid
MYB	Myeloblastosis
Nab	Nabutenne, Sri Lanka

NAC	NAM, ATAF, and CUC
NaDH	*Nicotianaattenuata* data hub
NADP	Nicotinamide adenine dinucleotide phosphate
NAM	No apical meristem
NAM	Nested association mapping
NattCyc	*N. attenuata*
NBS-LRR	Nucleotide-binding site-leucine-rich repeat
NCBI	National Center for Biotechnology Information
ncRNA	Non-coding RNA
ndhB	NADH dehydrogenase subunit
NE	North eastern
NF	Neurofibromin
NFYA	Nuclear transcription factor Y subunit alpha
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
NI	Neutral invertase
NIA2	Nitrate reductase
NILs	Near-isogenic lines
NIT1	Nitrilase 1
NMR	Nuclear magnetic resonance
NnCIPK	Calcineurin B-like protein kinase
NP	Nanoparticle
nptII	Neomycinephosphotransferase
NR	Natural rubber
nr	Non-redundant
Nrase	Nitrogen reductace
NRT	Nitrate transporter
NRT1.2	Nitrate transporter 1:2
NRT3.2	High-affinity nitrate transporter 3.2
NUE	Nitrogen use efficiency
OC	Oryzacystatin gene
ORF	Open reading frame
OsEBP2	*Oryza sativa* ethylene-responsive-element binding protein 2
OTC	Open top chamber
P5CS	Pyrroline-5-carboxylate synthetase
P5CSF129A	Δ1-Pyrroline-5-carboxylate synthetase
PAGE	Polyacrylamide gel electrophoresis
PAV	Presence/absence variant
PB	Prang Besar, Malaysia
PBR	Plant Breeders' Rights
PCD	Programmed cell dead
PCR	Polymerase chain reaction
PDH45	Pea DEAD-box helicase gene 45
PDS	Phytoene desaturase gene
PE	Physiological effectiveness

PEG	Polyethylene glycol
PGDBs	Pathway Genome Databases
PhERF	*Petuniahybrid* ethylene response factor
Pil	Pilmoor, Malaysia
PIN3	Polar auxin transport genes
PIR	Protein Information Resource
PN/Pn	Net Photosynthetic rate
POD	Peroxidase
PP2A	Protein phosphatase
PPB	Participatory plant breeding
ppm	Parts per million
PPO	Polyphenol oxidase
PR	Profestationvoor Rubber, Indonesia
pre-crRNA	Precursor CRISPR RNA
PRT	Progeny row trials
PS	Photosystem
psaB	Photosystem subunit
PSII	Photosystem II
PsTP	Pleurotussajor-cajutrehalose, phosphorylase
PVP	Plant Variety Protection
PX4	One class III peroxidase
PYT	Primary yield trail
QC	Quality checked
QTLs	Quantitative trait loci
QTL-seq	QTL sequencing
QTN	Quantitative trait nucleotide
RAD seq	Restriction-set associated DNA sequencing
RAD	Restriction site-associated DNA
RAMP	Random amplified microsatellite polymorphism
RAPD	Random/ly amplified polymorphic DNA
RAV	Related to ABI3 and VP1
RE	Recovery efficiency
REF	Rubber elongation factor
RFLP	Restriction fragment polymorphism
RFOs	Raffinose family oligosaccharides
RGT	Rapid generation turnover
RH	Relative humidity
RILs	Recombinant inbred lines
RNA	Ribonucleic acid
RNAi	RNA interference
RNPs	Ribonucleoproteins
ROS	Reactive oxygen species
RP	Recurrent parent
rp	Ribosomal protein
RRIC/RRISL	Rubber Research Institute of Sri Lanka

RRII	Rubber Research Institute of India
RRIM	Rubber Research Institute of Malaysia
RRIV	Rubber Research Institute of Vietnam
RS-GIS	Remote sensing and geographic information system
RT-qPCR	Reverse transcription-quantitate PCR
RuBPC	Ribulose-1,5-bisphosphate carboxylase
RWC	Relative water content
S1Z 1	Sumo ligase 1
SAH	Semi-autotrophic hydroponic
SALB	South American leaf blight
SBI	ICAR-Sugarcane Breeding Institute
SbpAPX	Peroxisomal ascorbate peroxidase
SCATC	South China Academy of Tropical Crops
ScCAT1	Sugarcane catalase 1
ScCBLs	Sugarcane casitas B-lineage lymphoma genes
ScNsLTPs	Sugarcane non-specific lipid transfer proteins
SCW	Silicon carbide whiskers
SDS	Sodium dodecyl sulfate
SFP	Single feature polymorphism
SG	Subgroup
SGN	Sol Genomics Networks
SHN1	SHINE 1 transcription factor
SIB	Swiss Institute of Bioinformatics
siRNAs	Short interfering RNAs
SLAF-seq	Specific length amplified fragment sequencing
SMRT	Single-molecule real-time sequencing
Snac1	Stress responsive nac1
SnoRNAs	Small nucleolar RNAs
SNP	Single nucleotide polymorphism
snRNAs	Small nuclear RNA
SOD	Superoxide dismutase
Solana Cyc	Solanaceae database
SPAD	Soil plant analysis development
SppRNAs	Short-proximal RNAs
SPS	Sucrose phosphate synthase
SRA	Sequence Read Archive
SRAP	Sequence-related amplified polymorphism
SRPP	Small rubber particle protein
SrRNA	Small rDNA-derived RNA
SRS-Seryl	tRNAsynthetase
SS	Sucrose synthase
SSAP	Sequence-specific amplification polymorphism
SSCP	Single-stranded conformation polymorphism
SSH	Suppression subtractive hybridization
SSLP	Simple sequence length polymorphism

SSNs	Sequence specific nucleases
SSR	Simple sequence repeat
STMS	Sequence tagged microsatellite
STR	Short tandem repeat
STRE	Stress response element
STS	Sequence tagged site
Suc	Sucrose
SUCEST	Sugarcane EST Genome Project
SV	Structural variant
SV1	Somaclonal variant-1
TAC	Transformation-competent artificial chromosome
TALEN	Transcription activator-like effector nuclease
TCTP	Transcription factor and translationally controlled tumor protein
TF	Transcription factor
TFL	TERMINAL FLOWER 1
TGI	Tobacco Genome Initiative
TIR	Transport inhibitor response
Tjir	Tjirandji, Indonesia
TK	Traditional knowledge
TM	Transmembrane
T_{max}	Maximum temperature
T_{min}	Minimum temperature
tmRNA	Transfer messenger RNA
TobEA	Tobacco Expression Atlas
TOBFAC	Tobacco transcription factor
TOFMS	Time of flight mass spetrometry
T_{opt}	Optimum temperature
TPA	Third party annotation
TPD	Tapping panel dryness
TPS1	Trehalose-6-phosphate synthase
Tr	Transpiration rate
tracrRNA	Trans-activating crRNA
TRAP	Target region amplification polymorphism
tRNA	Transfer RNA
TsRNA	tRNA-derived small RNA
TUB	Tubulin beta gene
U18S	18S ribosomal RNA
UAM	Universidad Autónoma de Manizales
UBQ	Ubiquitin
UBQ10	Polyubiquitin
UE	Utilization efficiency
UEMASUL	Universidade Estadual da Região Tocantina do Maranhão
UFES	Universidade Federal do Espírito Santo
ULISBOA	Universidade de Lisboa
UNFCCC	United Nations Framework Convention on Climate Change

UniParc	UniProt Archive
UniProt	Universal Protein Resource
UniProtKB	UniProt Knowledge Base
UniRef	UniProt Reference Clusters
UNL	Universidade NOVA de Lisboa
UNOESTE	Universidade do Oeste Paulista
UPOV	The International Union for the Protection of New Varieties of Plants
USDA	United State Department of Agriculture
UV	Ultraviolet
UV radiation	Ultraviolet radiation
UV-B	Ultaviolet B
VDE	Violaxanthin de-epoxidase
VIGS	Virus-induced gene silencing
Wagga	Wagga, Sri Lanka
War	Waringiana, Sri Lanka
WCSRG	World Collection of Sugarcane and Related Grasses
WGCNA	Weighted gene co-expression network analysis
WGRS	Whole-genome resequencing
WGS	Whole genome shotgun
WNTD	World No Tobacco Day
WOX	Wuschel like homeobOX
WRKY	W-box binding transcription factor
WUE	Water use efficiency
WUS	WUSCHEL gene
Y2H	Yeast-2-hybrid
YAC	Yeast artificial chromosome
YAD	Yam anthracnose disease
YMV	Yam mosaic virus
ZEA	Zeaxanthin
ZFNs	Zinc-finger nucleases

Chapter 1
Genomic Designing for Abiotic Stress-Resistant Cassava

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Abstract Several abiotic factors mostly drought, extreme temperatures, and high salinity negatively impact crop growth and productivity. As more fluctuations in these climatic elements are projected into the future, there is an urgent need to redesign breeding strategies to develop crop varieties that will withstand the impacts of these stressors. Cassava is an ideal stress-tolerant and staple crop grown around the regions of the world where the impacts of rising crop stressors will likely be felt more. To ensure continuous food production, and income generation to millions around the world, this chapter discusses the recent advances in understanding crop abiotic stress resistance mechanisms and the adoption of modern, especially genomics and phenotyping technologies in addressing abiotic stress tolerance in cassava. Although not in full use, the benefits of genetic engineering and genome editing in improving abiotic stress resistance in cassava were highlighted. Overall, the inclination and success in responding promptly to the demand for more tolerant and sustainable cassava rely on integrating various fields of studies in genomics, phenomics, transcriptomics, metabolomics, engineering, etc., using the rich germplasm resources in overcoming the limitations of conventional breeding technology to mitigate the impact of abiotic stressors.

Keywords Cassava · Abiotic stress · Genomics · Quantitative trait loci (QTL) · Breeding · Phenotyping

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1.1 Introduction

Cassava plays a significant role in global food security and provides affordable food and nutrition to millions. The crop is a valuable source of feed for livestock and is in high demand as a source of raw material in many industries. It has a unique starch quality that is desirable in many food, feed, textile, pharmaceutical, chemical, and biofuel industries and processes (Tonukari 2004; Zhang et al. 2016). In addition, the economic benefits and impact of cassava especially, in the low-income population is enormous. It is often regarded as a cash and food crop of resource-limited farmers in Africa, Asia, Latin America, and the Caribbean (Okogbenin et al. 2013). It stores so long in the soil after maturity and can be produced almost all year round in many parts of the world. When compared to other crops, cassava can withstand the advert effects of biotic and abiotic stressors (El-Sharkawy 2007; Okogbenin et al. 2013). It is about the only crop with a guaranteed relative high production under marginal, highly eroded, low-fertile, and acidic soils. In many low-scale productions, cassava is usually grown under minimal production inputs and high dependence on often erratic rainfall and almost zero budget on fertilizer application. The characteristic ability of the crop to thrive under marginal conditions, water stress, and poor soils with little to no addition of the basic agrochemical inputs, where many other crops might not survive are traceable to the high metabolic efficiency, energy per unit area production, and lack of specific water-stress sensitivity beyond storage root initiation (El-Sharkawy 1993; Okogbenin et al. 2013). In terms of yield potential, the energy production of cassava per hectare in environments with no production limitations matches or exceeds those of other major crops such as maize, sorghum, and rice (de Vries et al. 1967).

Despite this inherent high tolerance of cassava to many stress factors, a significantly higher performance under more favorable rainfall and soil fertility is possible, highlighting the massive yet untapped yield potential of the crop. Severe abiotic stress at critical growth period of the crop, especially, between 1 and 5 months after planting (MAP) which coincides with the stages of root initiation and tuberization, impacts the yield potential of the crop (Alves 2002; El-Sharkawy 2004). Such reduction in crop performance has been reported to be worse in grain and other crops around the globe (Wheeler and von Braun 2013; Raza et al. 2019). The increase in frequency, intensity and severity of these abiotic stresses will continue to negatively impact crops yield and impose a severe threat to global food security (Daryanto et al. 2016; Orek et al. 2020). Among other viable interventions, breeding techniques to improve known stress-tolerant staple crop varieties that can produce higher yields under adverse climatic conditions is a reliable alternative and cassava fits the ideal description of a stress-tolerant staple crop for such mitigation efforts. However, the research efforts in determining and improving stress-related traits that will help to promote the good performance of cassava under abiotic stress conditions have been slow.

The traditional improvement strategies for cassava until recently have relied on the phenotypic selection which takes a long time, is less accurate, and is labor-intensive.

Also, the accurate characterization of general and specific responses to particular or complex abiotic stressors at different physiological, morphological, etc., levels are further complicated by difficulties and lack of high throughput phenotyping alternatives. However, the exploration of available genetic resources, research collaborations, and the adoption of emerging tools and technologies provide an opportunity for the improvement of abiotic stress-resistant cassava varieties. There are enormous genetic resources available globally for cassava research and the adoption of emerging breeding and phenotyping techniques will facilitate the development of new varieties resilient to abiotic stressors and reduce the lengthy breeding cycle of the crop. The rising interests in modern sensing technologies and computer vision have several applications in cassava, especially for abiotic stress-resistant improvement. There are new opportunities presented by the adoption of new molecular tools in cassava breeding to increase breeding efficiency and promote the rapid identification and selection of genes or genomic regions necessary for the fast-paced genetic advances of especially complex traits. Advanced gene editing and genetic engineering tools are also important in improving the accuracy and precision of new variety development. Complementary techniques in phenomics, genomics, metabolomics, transcriptomics, etc., further offer new opportunities to improve our understanding of the response mechanisms of cassava to abiotic stress.

This brief chapter presents an overview of the ongoing efforts using both conventional and emerging technologies in developing cassava varieties that have the potential to withstand the recent erratic climatic pattern around the globe. Most of the cassava growing regions in the world especially in sub-Saharan Africa are most vulnerable to the impacts of climatic fluctuations and if the current trends continue, arid, semi-arid, and dry sub-humid areas are expected to expand into the more favorable agricultural areas. With the slow rate of technology development and the adoption of modern inputs in cassava production, the development of resilient varieties presents the most efficient opportunity to address food security and arrest economic crisis in these areas where the majority of the population are dominantly rain-fed farmers.

1.2 Complexity and Impact of Abiotic Stress in Cassava

Cassava is a common crop around the tropics and sub-tropics of Africa, Asia, and Latin America and is cultivated between the latitudes of 30° N and 30° S from sea level to above 2000 m above mean sea level (El-Sharkawy 2006; Anikwe and Ikenganyia 2018). From the more favorable rainforests and savannah regions, cassava production continues to expand to the Sahelian regions where other staples can hardly survive (Romanoff and Lynam 1992; El-Sharkawy 2004). However, abiotic stress factors are known to interfere with many morphological, biochemical, and physiological processes related to cassava growth and yield. As global fluctuations in climate continue to trigger the severity of abiotic stresses through increased changes in CO_2 concentration, temperature extremes, drought severity, and unpredictable

rainfall patterns, several crop processes will continue to be impeded and eventually lessen production and yield (Bates et al. 2008; Vahdati and Leslie 2013). With the rising adverse climatic conditions, many of the cassava producing regions continue to experience low and erratic precipitation, dry air, and high temperatures as well as soil nutrient deficiencies, poor photosynthetic activity, and photorespiration.

Under a prolonged adverse drought and waterlogging, a reduction in storage root yield as high as 60% and a significant reduction in growth and development processes including leaf formation, shoot yield, plant height, root development, dry matter, and starch contents are obvious (Lahai et al. 1999). Secondary metabolite (glycine betaine, proline, MDA, abscisic acid) accumulation, source, and sink ratio variations due to abiotic stress has been reported (Yadav et al. 2020). The predicted upsurge in drought, flooding, land degradation, high CO_2/O_2 concentration, and extreme temperature (heat/cold) associated with variable climate may continue to impact cassava yield potential leading to an untold food crisis, particularly in tropical and subtropical regions of the world. A potential yield up to 90 t/ha has been reported with improved cassava cultivars in experimental trials under reduced stress conditions but the actual global average yield continues to revolve around 12 t/ha and as low as 10 t/ha in some regions of the world (El-Sharkawy 2005).

Several environmental stresses often activate similar cell signaling pathways and cellular responses, such as the production of stress proteins, up-regulation of antioxidants, and accumulation of compatible solutes (Wang et al. 2003). Most often, abiotic stresses from drought, salinity, cold, heat and chemical pollution are often interconnected, and trigger off similar cellular damage and secondary degree stresses including osmotic and oxidative stress. Drought and/or salinization are known to lead to osmotic stress, disruption of homeostasis, and ion distribution in the cell (Serrano et al. 1999; Zhu 2001). Similarly, oxidative stress results from high temperature, salinity, or drought stress can denature the function and structure of proteins (Smirnoff 1998; Wang et al. 2003). The molecular, cellular, and physiological modifications in response to abiotic stresses are usually quantitative and require more than a simple approach in dissecting genes linked to these traits. A list of the identified abiotic stress traits in cassava is difficult to evaluate and is often carried out very late in the growth cycle of the crop making the adoption of large-scale breeding and improvement techniques for abiotic stress very difficult or impossible (Okogbenin et al. 2013). Cassava suffers from the impact of notable abiotic stressors.

1.2.1 Drought Stress

Generally, plants need water for survival and the transport of nutrients. Drought stress results from a brief or prolonged shortage of water quality or quantity available to plants. Under an optimal condition, cassava requires more than 1000 mm of rainfall annually for best performance, however, under the rain fed system, which is practiced by many cassava growers, the crop is subjected to prolonged periods of dry periods ranging from 3 to 5 months in many regions (Okogbenin et al. 2013; Anikwe and

Ikenganyia 2018). Drought affects the storage root development which can impact the supply chain for food and the income level of so many that depend on the crop for their livelihood. The critical water requirement of cassava is reported as the early development of cassava and yield losses of the storage root due to drought could be up to 32% and 60% (Connor et al. 1981; Brown et al. 2016). Generally, the impact of drought could be averted in plants through avoidance, tolerance, escape, recovery, or a complex combination of the mechanisms (Fang and Xiong 2014; Muiruri et al. 2021). Specifically, through the modification of morphological features to maintain enough water for regular physiological functions, drought avoidance under dry conditions is more prevalent in cassava (Luo 2010; Orek et al. 2020). Leaves stomata under drought incidence in cassava are often restricted to limit evaporation while maintaining the rate of photosynthesis and leaf potential under a long spell of water stress. In addition, there is a potential accumulation of epicuticular wax on leaves, reduction of canopy light interception through reduced rate of leaf formation, abscission of already formed leaves, leaf drooping, and the production of smaller-sized leaves (El-Sharkawy and Cock 1987). The elaborate fine root system that can penetrate up to 2 m below soil depth, distribution and content of the plant growth regulator, abscisic acid (ABA), and its association with leaf stomata conductance are also important drought tolerance mechanisms in cassava. However, the rapid accumulation of large amounts of ABA, cessation of leaf expansion and transpiration of water-deficient, and the overall ability of cassava to resist the adverse effects of drought can be affected by genotype composition, the timing and duration of the event (Alves and Setter 2004a, b).

1.2.2 Extreme Temperatures and Light Stresses

Extreme hot or cold temperatures affect the growth, development, and yield of cassava. The optimum temperature range of between 25 and 29 °C is ideal for cassava but can still show tolerance to deviations from this range (Jennings and Iglesias 2002; Allem 2002). However, a significant decline in growth and photosynthetic rates become obvious as soil temperatures exceed 40 °C or fall below 15 °C in various cassava genotypes (Okogbenin et al. 2013). Extreme cold stress impacts all cellular functions—cause injury and cellular damage, and alter metabolism processes leading to reduced plant growth and vascular discoloration. Also, extreme temperatures can promote susceptibility to other stress factors or the eventual death of the plant (Boansi and Zhang 2017).

On the contrary, elevated temperatures affect the rate of sexual development, and can cause drought stress due to increased water loss by transpiration or evaporation. High temperatures impact various cassava processes from seed germination, growth, and development, to triggering irreversible drought stress that can lead to death (Allem 2002). Besides affecting plant height and above-ground biomass, the temperature can affect tuber yield, as well as foliar and tuber chemistry (Brown et al. 2016).

Usually, 12 h day photoperiod with long days is ideal and favors shoot development whereas, shorter days encourage root development (Pushpalatha and Gangadharan 2020). Cassava adapts to shading (reduced solar radiation) by increasing its height through stem elongation and reduced leaf retention as observed from field studies (Cock et al. 1979; Fukai et al. 1984). Under shade conditions, there is evidence that both light quality and intensity could be altered leading to higher far-red (FR), but lower red and blue light distribution (Vandenbussche et al. 2005). Signals including Red:FR ratio, blue light, and total light intensity can induce shade-avoidance responses including stem and petiole elongation, and plants grow tall and spindly for sunlight competition at the expense of leaves development. In addition, shading can lead to an increase in the net photosynthetic rate while reducing stomatal conductance, transpiration rate, and intercellular CO_2 concentration in cassava (Ding et al. 2016).

1.2.3 Soil Stress

Soil-associated abiotic stress range from nutrient depletion related to low soil fertility, aluminum and/or manganese toxicity (acidity), salinity, mineral deficiency, etc., and these factors impact crop performance (Tattar 1989; Rao et al. 2016). The recent rise in salinization of major cropping lands is associated with rising sea levels and an increase in topsoil erosion, predominant in mostly the sub-Saharan regions (Munns and Tester 2008; Gleadow et al. 2016). While cassava can tolerate some degree of salinity as well as other soil-related stresses, biomass production and root systems could be adversely affected by salinity (Cruz et al. 2017). Generally, salinity occurs in arid and semi-arid areas where yearly evaporation and transpiration rates in plants are higher compared to precipitation volume (Yadav et al. 2020). Primary or secondary soil salinity could be triggered by a natural increase in the subsoil or external modification of soil contents from pollution, fertilizer residues, saline water for irrigation, etc. (Okogbenin et al. 2013). Potential impacts of high salt concentration on growth and development include cell growth inhibition and reduced metabolism.

1.3 Approaches in Genomic Designing of Abiotic Stress Tolerant Cassava

1.3.1 Genetic and Genomic Improvement Strategies

1.3.1.1 Conventional Breeding Strategies

To keep pace with the growing demand for nutritional and sustainable food in the face of changing climate, the conception of plant responses to the adverse effects of

unfavorable climatic conditions and the adoption of new strategies to develop sustainable technologies and products are very critical. The resilience of cassava to various stressors presents an opportunity that is yet to be harnessed. Traditional improvement strategy based on a phenotypic selection in cassava is complicated and relies on the lengthy growth cycle of the crop. The breeding pipeline has been described starting from the generation of crosses and initially planting in a seedling block to generate enough planting materials that would be evaluated across various successive stages in preliminary, advanced, and uniform yield trials (Ceballos et al. 2012; Wolfe et al. 2017). Cassava is highly heterozygous and every cassava progeny carries unique and distinct genetic information. Across the different evaluation stages, the number of genotypes evaluated diminishes as the plot sizes, number of replications, and trialing locations increase. The early breeding stages are often complicated with the difficulty in generating botanical seeds, multiplication of planting materials, and adoption of best strategies for discarding unwanted genetic materials. It could take up to 2–3 years to generate sufficient seeds from target progenitors because of flowering-related issues and the technicality of making crosses in cassava (Ceballos et al. 2004, 2015). On the other hand, the opportunity to include more genetic materials for evaluation and the use of expanded plot sizes, replications, and locations depend on the success of generating sufficient planting materials which is demanding for a crop with a very low propagation rate compared to other crops (Ceballos et al. 2012; Okogbenin et al. 2013). In addition to the low multiplication rate of cassava, planting materials for the next season are usually preserved in the field and the quality of the planting materials can be impacted by adverse biotic and abiotic pressures leading to low genotype performance or total loss of materials. Since phenotypic evaluations at the early stages of selection are based on non-replicated trials grown in a single location, the traditional scheme is prone to experimental errors. Difficulty in adopting best strategies for selection at the early breeding stages increases the potential of losing useful unselected genetic materials since selection is mostly based on a limited number of traits that often exclude quality traits assessment. Therefore, many limiting factors not limited to the lack of elite clones with useful genes in core breeding germplasm, the irregular flowering and seed set problem, low germination and multiplication rate, accumulation of deleterious mutations, heterozygosity, and allopolyploid nature of cassava render the conventional breeding strategy in cassava very inefficient in the modern dispensation (Byrne 1984; Kawano 2003; Ceballos et al. 2004; Ojulong et al. 2008; Wolfe et al. 2017). As a result, it is imperative to adopt new tools, strategies, and technologies to fast-track cassava improvement.

1.3.1.2 Advanced Molecular, Transgenic, Gene-Editing Strategies

Earlier attempts in utilizing molecular resources ranging from restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), and isoenzymes were promising in overcoming the bottlenecks associated with the traditional breeding method in cassava (Fregene et al.

1997; Mba et al. 2001; Okogbenin et al. 2006; Raji et al. 2009). With advances in technology and recent investment in cassava research (Foundation 2012; Bredeson et al. 2016), the availability of molecular resources with better resolution and genome-wide coverage contribute to reshaping the precision and the dynamics of improving cassava. The availability of high-density whole-genome markers is important in the adoption of state-of-the-art genome-wide strategies to further reduce the breeding cycle of cassava while improving the rate of genetic gain. It supports the efforts in dissecting associations between the widely distributed markers and variation in phenotypes within a family structure focusing on mostly recent recombination events in quantitative trait loci (QTL) mapping or within the context of a wider population in genome-wide association studies (GWAS). In marker-assisted selection (MAS), molecular markers in or near genes that affect the phenotype of interest can be deployed in selection. Genome-wide association studies capture hundreds of thousands or more variants in the genome and are ideal for testing common variants with small effect sizes compared to the traditional linkage mapping. Although, both GWAS and QTL mapping are useful in identifying regions of the chromosome that are associated with the phenotype of interest, higher resolution in identifying linkage disequilibrium in natural populations without necessarily any need to generate new crosses is possible in GWAS than QTL mapping.

Similarly, genomic selection (GS) is another genome-wide technique suitable for the improvement of complex traits controlled by multiple loci of small genetic effects without any prior understanding of the underlying QTL. It depends on genome-wide markers and statistics to estimate the breeding values of selection candidates, that have been genotyped, from a previously developed model using a training population that has both genotype and phenotype, and pedigree information, where available (Meuwissen et al. 2001; Goddard and Hayes 2007). The GS strategy has been revolutionizing animal and plant breeding schemes including cassava, although the practical application for abiotic stress improvement in cassava is still scanty (Goddard 2009; Lorenz et al. 2011; Wolfe et al. 2017). The adoption of genome-wide tools is suitable for improving mostly the complex abiotic tolerant-associated traits in cassava. The application of GS relies on accurate historic information which is available in many national and international cassava breeding programs in North and South America (USA, Brazil, Columbia), Asia, West (Nigeria, Ghana), and East Africa (Tanzania, Uganda, etc.). The abundant germplasm records and genomic resources have been pivotal in the recent breakthroughs in cassava improvement. Most of the germplasms have been evaluated for pest and disease resistance and other economic traits and are useful in developing modern strategies in cassava improvement (Bellotti 2002; Okogbenin et al. 2007; Parkes 2011; Tumwegamire et al. 2018; Ogbonna et al. 2021). Highlights of recent efforts to ensure scalable, cost-effective, and optimized molecular resources in MAS for mapping QTL associated with abiotic stress and other traits include the use of genome-wide polymorphic KASParSNP markers within a 276 F_1 progeny in the dry savannah of Nigeria (Ewa et al. 2021). Strategies for integrating molecular markers to speed up cassava breeding and improve the accuracy of selection useful for abiotic stress tolerance are being established and are related to model selection, training population optimization, multiple trait prediction, selection

strategies, and efficient genotyping (Akdemir et al. 2015; Wolfe et al. 2017; Ikeogu et al. 2019; Okeke et al. 2017; Ozimati et al. 2018; Somo et al. 2020).

As the field of molecular genetics continues to advance, transgenic approaches have enabled the manipulations of the genes regulating transcription factors, antioxidants, protective proteins, transporters, membrane composition, etc., with better precision (Anwar and Kim 2020; Singh et al. 2013). Genetic engineering of the regulatory genes for controlling the expression of many stress-responsive genes offers a greater advantage over conventional breeding approaches given the complexity of stress-tolerant traits that limits the success of conventional breeding methods. There are notable engineering breakthroughs from the literature that are yet to be fully explored in cassava but are relevant in future cassava research, especially for abiotic stress improvement. Several genes including genes encoding enzymes for the biosynthesis of compatible compounds, enzymes for scavenging active oxygen species, heat shock proteins (HSPs), late embryogenesis-abundant (LEA) proteins, enzymes modifying membrane lipid saturation, transcription factors, and proteins required for ion homeostasis have been targeted in many studies for improving abiotic stress resistance (Hare et al. 1998; Zhang et al. 2000; Sun et al. 2020). The overexpression of the peroxisomal ascorbate peroxidase (SbpAPX) gene cloned from halophyte *Salicornia brachiate* was able to improve salt and drought stress tolerance in tobacco (Singh et al. 2013; Sun et al. 2020); overexpression of *OsEREBP1*, an AP2/ERF transcription factor conferred multiple abiotic and biotic stress tolerance in rice (Singh et al. 2013); increased tolerance to salt stress sweet potato (Sun et al. 2020); drought stress tolerance through ABA-dependent pathway (Shan et al. 2022); and enhanced tolerance against drought, salinity and cold stress (Checker et al. 2011). Since responses of plants to abiotic stresses are multigenic, therefore, the adoption of approaches beyond a single gene manipulation will ensure the stimulation of multiple cascades of cellular changes required for developing stress-tolerant plants. The simultaneous expression of downstream stress-inducible genes and parallel increases in stress tolerance could be achieved by identifying, cloning, and genetic engineering complex metabolic or regulatory pathways involving multiple genes encoding for abiotic stress (dos Reis et al. 2018). In addition to the simultaneous transfer of multiple genes by transformation with multiple genes or by crossing plants containing different stress tolerance genes, tolerance to several stresses is possible by utilizing regulatory gene inducing multiple target genes or of a single gene having multiple stress-protective effects (Zhang et al. 2000).

Also, genome editing techniques, such as CRISPR-Cas9, TALENs, ZFNs, have been gaining attention in crop improvement. Editing provides an opportunity for genetic modifications and is comparatively easy, fast, efficient, and accurate in developing new materials for enhanced abiotic stress tolerance (Garneau et al. 2010; Ran et al. 2013). Using CRISPR/Cas9 binary vector set and a gRNA module vector set, a multi-genome editing toolkit has been developed which fosters the use of CRISPR/Cas9 in a variety of plant systems and is especially useful for high-efficiency multiplex plant genome editing (Hameed et al. 2018). The record of genome-editing in cassava is scanty but there are a few references that show the potential of adopting the technique in cassava to improve many important traits. Integrated CRISPR/Cas9

and gRNA was recently used in genome editing of *Phytoene desaturase* in cassava with considerable success, although specific modifications are still needed for a wider application, considering the high level of heterozygosity and inbreeding depression associated with the crop (Odipio et al. 2017; Gomez et al. 2019, 2021).

1.3.2 Enhanced Phenotyping

To sustain steady genetic gain in abiotic stress improvement in cassava, setbacks associated with large-scale and accurate phenotyping of responses to abiotic stress must be addressed. Some of the conventional abiotic stress traits including storage root features are carried out very late in the growth stage of the plant, are destructive and tedious for large-scale experiments. To complement the recent advances in genotyping technologies, phenotyping is essential in designing sustainable strategies to respond to the urgent demand for new varieties of cassava in this era of increasing fluctuations in climatic conditions. Several opportunities exist in computer vision, and sensing technologies for rapid, non-destructive, and accurate assessment of abiotic traits in cassava (Kim et al. 2011; Ikeogu et al. 2017; Ramcharan et al. 2017; Rocha Bessa De Carvalho et al. 2022). Recently, the field of spectroscopy which examines electromagnetic radiation and its production from, or its interaction with matter has been valuable in crop phenomics (Pasquini 2003; Stuart 2004; Ge et al. 2019). It has a wide application in quantitative and qualitative studies. The fundamental principle of spectroscopy on the unique spectral output of every structural substance could be used to understand the structure of the substance or determine its chemical composition. Either for imaging or non-imaging, contact or remotely, the most common types of spectroscopies include visible wavelength spectroscopy (VIS), near-infrared resonance (NIR), short-wave infrared wavelength (SWIR), nuclear magnetic resonance (NMR), and selected ion flow tube mass spectroscopy (SIFT-MS). Currently, progress has been recorded in deploying spectroscopy technology as an analytical tool for evaluating the quality constituents of cassava storage roots (Sánchez et al. 2014; Belalcazar et al. 2016; Ikeogu et al. 2017, 2019). Compared to the traditional phenotyping methods, they are fast, less destructive, cost-effective, and applicable for simultaneous quantification or classification of multiple traits.

Besides the evaluation of root-related traits for abiotic studies, spectroscopy offers an opportunity to study the structural and chemical responses of cassava to various abiotic factors at other tissues. Combined with imaging tools, it is now easier to capture in real-time, specific responses to abiotic stress now than before. Our ongoing effort is focusing on dissecting leaf-spectral information to enable the understanding of useful variations in structure and responses to stressors that could be used separately or integrated with the current MAS to further improve the accuracy of breeding (ongoing research). Profiling the seasonal dynamics of leaf traits under field conditions using spectroscopy will help overcome the previous dearth in phenotyping and consequent genomic improvement of abiotic tolerance in cassava. More opportunities exist in metabolites fingerprinting and discrimination in response to abiotic

stress using NMR. Also, thermal-based imaging spectroscopy sensors are excellent for precise spatial measurement of tissue-specific or canopy temperature evaluation in response to drought, nutrient deficiency, and other stressors. The advantage is that many plots can be evaluated simultaneously in field trials, allowing for a comprehensive comparison of differences in canopy temperature among genotypes (Jones et al. 2009). Also, the image outputs from dual-purpose spectrometers or single imaging cameras when combined with artificial intelligence (AI) technologies are useful in assessing the morphological and developmental phenotypes associated with abiotic stress in real-time (Manley 2014; Mishra et al. 2017; Khan et al. 2018; Bauer et al. 2019).

1.4 Conclusion

Although cassava can tolerate most abiotic stress, genetic improvement strategies can be adopted to enhance productivity and maximize the potential yield of the crop. An urgent gap in genomic designing of tolerant cassava to abiotic stress exists in the accurate quantification responses to these abiotic stressors. The conventional breeding strategy in cassava is laborious; time-consuming and unsuitable for improving most of the complex, quantitative and multigenic traits associated with abiotic stress. However, the advancement in various fields offers opportunities that are yet to be fully maximized in cassava. Rather than depending on a single strategy, efforts in optimizing and integrating concepts phenomics, genomics, transcriptomics, metabolomics, and computation will help to speed up and enhance the accuracy and efficiency of abiotic stress tolerance in cassava.

Integrating the recent innovations into breeding strategies will help decipher the stress signaling pathways and the development of strategies to ensure faster genetic gains than is possible through conventional breeding. Current access to whole-genome information on cassava and their wild relatives to be effective in rapid adaptation to climate. Ongoing efforts in machine learning and computation, data management and software developments will further contribute to breakthroughs in abiotic stress improvement in cassava.

References

Akdemir D, Sanchez JI, Jannink JL (2015) Optimization of genomic selection training populations with a genetic algorithm. Genet Sel Evol 47. https://doi.org/10.1186/s12711-015-0116-6

Allem AC (2002) Chapter 1. The origins and taxonomy of cassava. In: Hillocks RJ, Thresh JM (eds) Cassava: biology, production and utilization. CABI, Wallingford, pp 1–16

Alves A (2002) Chapter 5. Cassava botany and physiology. In: Hillocks RJ, Thresh JM (eds). CABI Pub, pp 67–89

Alves AAC, Setter TL (2004a) Response of cassava leaf area expansion to water deficit: cell proliferation, cell expansion and delayed development. Ann Bot 94:605. https://doi.org/10.1093/AOB/MCH179

Alves AAC, Setter TL (2004b) Abscisic acid accumulation and osmotic adjustment in cassava under water deficit. Environ Exp Bot 51:259–271. https://doi.org/10.1016/J.ENVEXPBOT.2003.11.005

Anikwe MAN, Ikenganyia EE (2018) Ecophysiology and production principles of cassava (Manihot species) in Southeastern Nigeria. Cassava. https://doi.org/10.5772/INTECHOPEN.70828

Anwar A, Kim J-K (2020) Molecular sciences transgenic breeding approaches for improving abiotic stress tolerance: recent progress and future perspectives. Int J Mol Sci 21(8):2695. https://doi.org/10.3390/ijms21082695

Bates BC, Kundzewicz ZW, Wu S, Palutikof JP (2008) Climate change and water. Technical paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat. Intergovernmental Panel on Climate Change, Geneva

Bauer A, Bostrom AG, Ball J et al (2019) Combining computer vision and deep learning to enable ultra-scale aerial phenotyping and precision agriculture: a case study of lettuce production. Hort Res 6:1–12. https://doi.org/10.1038/s41438-019-0151-5

Belalcazar J, Dufour D, Andersson MS et al (2016) High-throughput phenotyping and improvements in breeding cassava for increased carotenoids in the roots. Crop Sci 56:2916–2925. https://doi.org/10.2135/cropsci2015.11.0701

Bellotti AC (2002) Arthropod pests. In: Hillocks RJ, Thresh JM (eds) Cassava: biology, production and utilization. CABI, Wallingford, pp 209–235

Boansi D, Zhang Y (2017) Effect of climatic and non-climatic factors on cassava yields in Togo: agricultural policy implications. Climate 5:28. https://doi.org/10.3390/CLI5020028

Bredeson JV, Lyons JB, Prochnik SE et al (2016) Sequencing wild and cultivated cassava and related species reveals extensive interspecific hybridization and genetic diversity. Nat Biotechnol 34:562–570. https://doi.org/10.1038/nbt.3535

Brown AL, Cavagnaro TR, Gleadow R, Miller RE (2016) Interactive effects of temperature and drought on cassava growth and toxicity: implications for food security? Glob Change Biol 22:3461–3473. https://doi.org/10.1111/GCB.13380

Byrne D (1984) Breeding cassava. In: Janick J (ed) Plant breeding reviews. Wiley, Hoboken, NJ, pp 73–134

Ceballos H, Iglesias CA, Pérez JC, Dixon AGO (2004) Cassava breeding: opportunities and challenges. Plant Mol Biol 56:503–516. https://doi.org/10.1007/s11103-004-5010-5

Ceballos H, Kulakow P, Hershey C (2012) Cassava breeding: current status, bottlenecks and the potential of biotechnology tools. Trop Plant Biol 5:73–87. https://doi.org/10.1007/s12042-012-9094-9

Ceballos H, Kawuki RS, Gracen VE et al (2015) Conventional breeding, marker-assisted selection, genomic selection and inbreeding in clonally propagated crops: a case study for cassava. Theor Appl Genet 128:1647–1667. https://doi.org/10.1007/s00122-015-2555-4

Checker VG, Chhibbar AK, Khurana P (2011) Stress-inducible expression of barley Hva1 gene in transgenic mulberry displays enhanced tolerance against drought, salinity and cold stress. Transgen Res 21(5):939–957. https://doi.org/10.1007/S11248-011-9577-8

Cock JH, Franklin D, Sandoval G, Juri P (1979) The ideal cassava plant for maximum yield. Crop Sci 19(2):271–279. https://doi.org/10.2135/CROPSCI1979.0011183X001900020025X

Connor DJ, Cock JH, Parra GE (1981) Response of cassava to water shortage I. Growth and yield. Field Crops Res 4:181–200. https://doi.org/10.1016/0378-4290(81)90071-X

Cruz JL, Filho MAC, Coelho EF, dos Santos AA (2017) Salinity reduces carbon assimilation and the harvest index of cassava plants (Manihot esculenta Crantz). Acta Scientiarum Agron 39:545–555. https://doi.org/10.4025/ACTASCIAGRON.V39I4.32952

Daryanto S, Wang L, Jacinthe PA (2016) Global synthesis of drought effects on maize and wheat production. PLoS ONE 11:e0156362. https://doi.org/10.1371/JOURNAL.PONE.0156362

de Vries CA, Ferwerda JD, Flach M (1967) Choice of food crops in relation to actual and potential production in the tropics. NL J Agri Sci 15. https://doi.org/10.18174/njas.v15i4.17429

Ding Z, Zhang Y, Xiao Y et al (2016) Transcriptome response of cassava leaves under natural shade. Sci Rep 6:1–14. https://doi.org/10.1038/srep31673

dos Reis SP, Marques DN, Ferreira Barros NL et al (2018) Genetically engineered food crops to abiotic stress tolerance. Genet Eng Foods 6:247–279. https://doi.org/10.1016/B978-0-12-811519-0.00010-8

El-Sharkawy MA (1993) Drought-tolerant cassava for Africa, Asia, and Latin America. Bioscience 43:441–451. https://doi.org/10.2307/1311903

El-Sharkawy MA (2004) Cassava biology and physiology. Plant Mol Biol 56:481–501. https://doi.org/10.1007/S11103-005-2270-7

El-Sharkawy AM (2005) How can calibrated research-based models be improved for use as a tool in identifying genes controlling crop tolerance to environmental stresses in the era of genomics-from an experimentalist's perspective. 43:161–176. https://doi.org/10.1007/S11099-005-0030-1

El-Sharkawy MA (2006) International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. 44:481–512. https://doi.org/10.1007/S11099-006-0063-0

El-Sharkawy MA (2007) Physiological characteristics of cassava tolerance to prolonged drought in the tropics: implications for breeding cultivars adapted to seasonally dry and semiarid environments. Braz J Plant Physiol 19:257–286. https://doi.org/10.1590/S1677-04202007000400003

El-Sharkawy MA, Cock JH (1987) Response of cassava to water stress. Plant Soil 100:345–360. https://doi.org/10.1007/BF02370950

Ewa F, Asiwe JNA, Okogbenin E et al (2021) KASPar SNP genetic map of cassava for QTL discovery of productivity traits in moderate drought stress environment in Africa. Sci Rep 11. https://doi.org/10.1038/s41598-021-90131-8

Fang Y, Xiong L (2014) General mechanisms of drought response and their application in drought resistance improvement in plants. Cell Mol Life Sci 72:673–689. https://doi.org/10.1007/S00018-014-1767-0

Foundation MG (2012) Next generation cassava breeding

Fregene M, Angel F, Gomez R et al (1997) A molecular genetic map of cassava (Manihot esculenta Crantz). Theor Appl Genet 95:431–441

Fukai S, Alcoy AB, Llamelo AB, Patterson RD (1984) Effects of solar radiation on growth of cassava (Manihot esculenta crantz.). I. Canopy development and dry matter growth. Field Crops Res 9:347–360. https://doi.org/10.1016/0378-4290(84)90038-8

Garneau JE, Dupuis MÈ, Villion M et al (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468:67–71. https://doi.org/10.1038/nature09523

Ge Y, Atefi A, Zhang H et al (2019) High-throughput analysis of leaf physiological and chemical traits with VIS-NIR-SWIR spectroscopy: a case study with a maize diversity panel. Plant Methods 15:66. https://doi.org/10.1186/s13007-019-0450-8

Gleadow R, Pegg A, Blomstedt CK (2016) Resilience of cassava (Manihot esculenta Crantz) to salinity: implications for food security in low-lying regions. J Exp Bot 67:5403. https://doi.org/10.1093/JXB/ERW302

Goddard M (2009) Genomic selection: prediction of accuracy and maximisation of long term response. Genetica 136:245–257. https://doi.org/10.1007/s10709-008-9308-0

Goddard ME, Hayes BJ (2007) Genomic selection. J Anim Breed Genet 124:323–330. https://doi.org/10.1111/j.1439-0388.2007.00702.x

Gomez MA, Lin ZD, Moll T et al (2019) Simultaneous CRISPR/Cas9-mediated editing of cassava eIF4E isoforms nCBP-1 and nCBP-2 reduces cassava brown streak disease symptom severity and incidence. Plant Biotechnol J 17:421–434. https://doi.org/10.1111/PBI.12987

Gomez MA, Berkoff KC, Gill BK et al (2021) CRISPR-Cas9-mediated knockout of CYP79D1 and CYP79D2 in cassava attenuates toxic cyanogen production. bioRxiv 2021.10.08.462827. https://doi.org/10.1101/2021.10.08.462827

Hameed A, Zaidi SS, Shakir S, Mansoor S (2018) Applications of new breeding technologies for potato improvement. Front Plant Sci 9:925. https://doi.org/10.3389/FPLS.2018.00925/BIBTEX

Hare PD, Cress WA, van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21

Ikeogu UN, Davrieux F, Dufour D et al (2017) Rapid analyses of dry matter content and carotenoids in fresh cassava roots using a portable visible and near infrared spectrometer (Vis/NIRS). PLoS ONE 12:e0188918. https://doi.org/10.1371/journal.pone.0188918

Ikeogu UN, Akdemir D, Wolfe MD et al (2019) Genetic correlation, genome-wide association and genomic prediction of portable NIRS predicted carotenoids in cassava roots. Front Plant Sci 10:1570. https://doi.org/10.3389/fpls.2019.01570

Jennings DL, Iglesias C (2002) Cassava: biology, production and utilization. CABI, Wallingford

Jones HG, Serraj R, Loveys BR et al (2009) Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Funct Plant Biol 36:978–989. https://doi.org/10.1071/FP09123

Kawano K (2003) Thirty years of cassava breeding for productivity—biological and social factors for success. Crop Sci 43:1325. https://doi.org/10.2135/cropsci2003.1325

Khan MJ, Khan HS, Yousaf A et al (2018) Modern trends in hyperspectral image analysis: a review. IEEE Access 6:14118–14129

Kim Y, Glenn DM, Park J et al (2011) Hyperspectral image analysis for water stress detection of apple trees. Comput Elect Agri 77:155–160. https://doi.org/10.1016/j.compag.2011.04.008

Lahai MT, George JB, Ekanayake IJ (1999) Cassava (Manihot esculenta Crantz) growth indices, root yield and its components in Upland and Inland Valley ecologies of Sierra Leone. J Agron Crop Sci 182:239–248. https://doi.org/10.1046/J.1439-037X.1999.00299.X

Lorenz AJ, Chao S, Asoro FG et al (2011) Genomic selection in plant breeding. Knowledge and prospects. Academic Press

Luo LJ (2010) Breeding for water-saving and drought-resistance rice (WDR) in China. J Exp Bot 61:3509–3517. https://doi.org/10.1093/jxb/erq185

Manley M (2014) Near-infrared spectroscopy and hyperspectral imaging: non-destructive analysis of biological materials. Chem Soc Rev 43:8200–8214. https://doi.org/10.1039/C4CS00062E

Mba REC, Stephenson P, Edwards K et al (2001) Simple sequence repeat (SSR) markers survey of the cassava (Manihot esculenta Crantz) genome: towards an SSR-based molecular genetic map of cassava. Theor Appl Genet 102:21–31. https://doi.org/10.1007/S001220051614

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

Mishra P, Asaari MSM, Herrero-Langreo A et al (2017) Close range hyperspectral imaging of plants: a review. Biosyst Eng 164:49–67

Muiruri SK, Ntui VO, Tripathi L, Tripathi JN (2021) Mechanisms and approaches towards enhanced drought tolerance in cassava (Manihot esculenta). Curr Plant Biol 28:100227. https://doi.org/10.1016/J.CPB.2021.100227

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Odipio J, Alicai T, Ingelbrecht I et al (2017) Efficient CRISPR/cas9 genome editing of phytoene desaturase in cassava. Front Plant Sci 8:1780. https://doi.org/10.3389/FPLS.2017.01780/BIBTEX

Ogbonna AC, Braatz de Andrade LR, Mueller LA et al (2021) Comprehensive genotyping of a Brazilian cassava (Manihot esculenta Crantz) germplasm bank: insights into diversification and domestication. Theor Appl Genet 134:1343–1362. https://doi.org/10.1007/s00122-021-03775-5

Ojulong H, Labuschangne MT, Fregene M, Herselman L (2008) A cassava clonal evaluation trial based on a new cassava breeding scheme. Euphytica 160:119–129. https://doi.org/10.1007/s10681-007-9590-4

Okeke UG, Akdemir D, Rabbi I et al (2017) Accuracies of univariate and multivariate genomic prediction models in African cassava. Genet Select Evol 49:88. https://doi.org/10.1186/s12711-017-0361-y

Okogbenin E, Marin J, Fregene M (2006) An SSR-based molecular genetic map of cassava. Euphytica 147:433–440. https://doi.org/10.1007/s10681-005-9042-y

Okogbenin E, Porto MCM, Egesi C et al (2007) Marker-assisted introgression of resistance to cassava mosaic disease into Latin American germplasm for the genetic improvement of cassava in Africa. Crop Sci 47. https://doi.org/10.2135/cropsci2006.10.0688

Okogbenin E, Setter TL, Ferguson M et al (2013) Phenotypic approaches to drought in cassava: review. Front Physiol 4. https://doi.org/10.3389/FPHYS.2013.00093/ABSTRACT

Orek C, Gruissem W, Ferguson M, Vanderschuren H (2020) Morpho-physiological and molecular evaluation of drought tolerance in cassava (Manihot esculenta Crantz). Field Crops Res 255:107861. https://doi.org/10.1016/J.FCR.2020.107861

Ozimati A, Kawuki R, Esuma W et al (2018) Training population optimization for prediction of cassava brown streak disease resistance in West African Clones. G3: Genes Genomes Genet 8. https://doi.org/10.1534/g3.118.200710

Parkes EY (2011) Assessment of genetic diversity, combining ability, stability and farmer preference of cassava germplasm in Ghana. https://scholar.ufs.ac.za/handle/11660/1453

Pasquini C (2003) Near infrared spectroscopy: fundamentals, practical aspects and analytical applications. J Braz Chem Soc 14:198–219. https://doi.org/10.1590/S0103-50532003000200006

Pushpalatha R, Gangadharan B (2020) Is cassava (Manihot esculenta Crantz) a climate "smart" crop? A review in the context of bridging future food demand gap. Trop Plant Biol 13:201–211. https://doi.org/10.1007/S12042-020-09255-2

Raji AA, Anderson JV, Kolade OA et al (2009) Gene-based microsatellites for cassava (Manihot esculenta Crantz): prevalence, polymorphisms, and cross-taxa utility. BMC Plant Biol 9:1–11. https://doi.org/10.1186/1471-2229-9-118/TABLES/3

Ramcharan A, Baranowski K, McCloskey P et al (2017) Deep learning for image-based cassava disease detection. Front Plant Sci 8:1852. https://doi.org/10.3389/FPLS.2017.01852/BIBTEX

Ran FA, Hsu PD, Wright J et al (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc 8(11):2281–2308. https://doi.org/10.1038/nprot.2013.143

Rao IM, Miles JW, Beebe SE, Horst WJ (2016) Root adaptations to soils with low fertility and aluminium toxicity. Ann Bot 118:593. https://doi.org/10.1093/AOB/MCW073

Raza A, Razzaq A, Mehmood SS et al (2019) Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. Plants 8. https://doi.org/10.3390/PLANTS8020034

Rocha Bessa De Carvalho R, Fernando D, Cortes M et al (2022) Image-based phenotyping of cassava roots for diversity studies and carotenoids prediction. PLoS ONE 17:e0263326. https://doi.org/10.1371/JOURNAL.PONE.0263326

Romanoff S, Lynam J (1992) Commentary. Cassava and african food security: some ethnographic examples. Ecol Food Nutr 27. https://doi.org/10.1080/03670244.1992.9991223

Sánchez T, Ceballos H, Dufour D et al (2014) Prediction of carotenoids, cyanide and dry matter contents in fresh cassava root using NIRS and Hunter color techniques. Food Chem 151:444–451. https://doi.org/10.1016/j.foodchem.2013.11.081

Serrano R, Mulet JM, Rios G et al (1999) A glimpse of the mechanisms of ion homeostasis during salt stress. J Exp Bot 50:1023–1036. https://doi.org/10.1093/JXB/50.SPECIAL_ISSUE.1023

Shan YJ, Li D, Cao JJ et al (2022) Over-expression of Arabidopsis ORANGE gene enhances drought stress tolerance through ABA-dependent pathway in Arabidopsis thaliana. Plant Growth Regul 96:91–101. https://doi.org/10.1007/S10725-021-00760-2

Singh N, Mishra A, Jha B (2013) Over-expression of the peroxisomal ascorbate peroxidase (SbpAPX) gene cloned from halophyte Salicornia brachiata confers salt and drought stress tolerance in transgenic tobacco. Marine Biotechnol 16:321–332. https://doi.org/10.1007/S10126-013-9548-6

Smirnoff N (1998) Plant resistance to environmental stress. Curr Opin Biotechnol 9:214–219. https://doi.org/10.1016/S0958-1669(98)80118-3

Somo M, Kulembeka H, Mtunda K et al (2020) Genomic prediction and quantitative trait locus discovery in a cassava training population constructed from multiple breeding stages. Crop Sci 60. https://doi.org/10.1002/csc2.20003

Stuart BH (2004) Infrared spectroscopy: fundamentals and applications. Wiley

Sun H, Sun X, Wang H, Ma X (2020) Advances in salt tolerance molecular mechanism in tobacco plants. Hereditas 157:1–6. https://doi.org/10.1186/S41065-020-00118-0/FIGURES/1

Tattar TA (1989) Soil stress. Dis Shade Trees 250–263. https://doi.org/10.1016/B978-0-12-684351-4.50023-5

Tonukari N (2004) Cassava and the future of starch. Elect J Biotechnol 7:5–8. https://doi.org/10.4067/S0717-34582004000100003

Tumwegamire S, Kanju E, Legg J et al (2018) Exchanging and managing in-vitro elite germplasm to combat cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) in Eastern and Southern Africa. Food Secur 10:351–368. https://doi.org/10.1007/s12571-018-0779-2

Vahdati K, Leslie C (2013) Abiotic stress—plant responses and applications in agriculture. https://doi.org/10.5772/45842

Vandenbussche F, Pierik R, Millenaar FF et al (2005) Reaching out of the shade. Curr Opin Plant Biol 8:462–468. https://doi.org/10.1016/J.PBI.2005.07.007

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14. https://doi.org/10.1007/S00425-003-1105-5/TABLES/1

Wheeler T, von Braun J (2013) Climate change impacts on global food security. Science 341:508–513. https://doi.org/10.1126/SCIENCE.1239402

Wolfe MD, del Carpio DP, Alabi O et al (2017) Prospects for genomic selection in cassava breeding. Plant Genome 10. https://doi.org/10.3835/plantgenome2017.03.0015

Yadav S, Modi P, Dave A et al (2020) Effect of abiotic stress on crops. Sustain Crop Prod. https://doi.org/10.5772/INTECHOPEN.88434

Zhang J, Klueva NY, Wang Z et al (2000) Genetic engineering for abiotic stress resistance in crop plants. In Vitro Cell Dev Biol Plant 36(2):108–114

Zhang M, Xie L, Yin Z et al (2016) Biorefinery approach for cassava-based industrial wastes: current status and opportunities. Bioresour Technol 215:50–62. https://doi.org/10.1016/j.biortech.2016.04.026

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 6:66–71. https://doi.org/10.1016/S1360-1385(00)01838-0

Chapter 2
Genomic Designing for Abiotic Stress Resistance in Coconut

S. V. Ramesh, V. Arunachalam, Semíramis Rabelo Ramalho Ramos, K. S. Muralikrishna, S. Paulraj, and M. K. Rajesh

Abstract Abiotic stressors associated with the climate change phenomenon are a serious threat to crop cultivation, especially in perennials like coconut (*Cocos nucifera* L.). In this challenging scenario, genomics-based crop improvement strategies are warranted to expedite the development of varieties resistant/tolerant to abiotic stresses and to enhance the rate of genetic gains. The genomic design of abiotic stress tolerance in coconut has not progressed as desired due to multiple challenges, including its perennial nature, breeding behavior, high heterozygosity, and long gestation period. Nonetheless, developments in the field of next-generation breeding approaches involving low-cost sequencing technologies, genotyping-by-sequencing, big-data analysis tools, and other RNA or protein-based genome-wide analyses have offered enormous prospects to design climate-smart coconut capable of withstanding most of the abiotic stresses. This chapter provides a perspective outlook on abiotic stress in coconut, salient accomplishments in adopting various genomic tools to develop stress-tolerant genotypes and a way forward.

Keywords Climate change · Climate-smart · Coconut · Genetic resources · Multi-omics data · Water-use efficiency

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2.1 Introduction

Coconut (*Cocos nucifera* L.) is a monotypic genus and an economically important palm linked to the socio-economic fabric of South, South-East Asia, and the Pacific Islands due to its multitude of uses. The palm is aptly revered as "*Kalpavriksha*" or "tree of heaven" because of its versatile utilities such as food, fiber, feed, medicine, etc. (Ramesh et al. 2021a). The palm is generally grown in the regions between 23° N and 23° S latitudes (Kumar and Aggarwal 2013); however, the crop could be cultivated at 26° N latitude. The temperature conditions that severely limit the growth of palm beyond the north of this latitude is a serious concern (Kumar and Aggarwal 2013).

Coconut is grown in more than 90 countries in the tropical regions of Asia, America, and East Africa. Currently, the coconut production in the world is estimated at 68,833 million nuts from 12.08 million ha and productivity of 5,777 nuts ha^{-1} (ICC 2019). Major producers, such as Indonesia and the Philippines, account for around 56% of the global coconut production, followed by India (Burton 2019). Coconut is endowed with great genetic variability, and hence huge variations exist in its morpho-physiological and biochemical characteristic features. Moreover, the uses of the palm in diverse fields such as the food industry, nutraceuticals and medicines, due to its unique fatty acid composition in the endosperm, antimicrobial properties, nutrient richness of nut water and inflorescence sap, etc., have renewed further interests in crop improvement programs.

Notwithstanding the economic and ecological potential of this crop, the true genetic potential of the palm has not been realized. There are numerous hurdles such as poor availability of quality planting materials, limited true to type progeny seed nuts, the influence of biotic and abiotic stressors, and other economic factors such as fluctuating prices for the coconut-based commodities, which contribute to reduced production potential (Buschena and Perloff 1991). Coconut requires characteristic tropical weather parameters for its luxuriant growth and economic yield. Evenly distributed precipitation of 130–230 cm $year^{-1}$, ample sunlight of 250–350 Wm^{-2} and a sunshine period of 120 h $month^{-1}$, along with annual mean temperature (27 °C) and relative humidity over 60% are the favorable climatic conditions for its optimal growth and yield (Child 1974; Murray 1977).

The wild relatives of coconut are not known, and hence the coconut accessions are inter-bred among themselves to develop improved cultivars. Molecular studies have unravelled that both Indian Ocean and Pacific Ocean basins have independently witnessed coconut domestication events (Gunn et al. 2011). Systematic breeding efforts in coconut involve germplasm collection to enrich the gene pool and conventional breeding approaches such as selection and hybridization (Arunachalam and Rajesh 2008, 2017). Despite the requirements of huge resources of land, labor, and technical expertise in pursuing scientific breeding efforts in coconut, improved cultivars with specific traits and enhanced productivity have been developed (Nair et al. 2016). A few of the successful instances of human intervention in coconut improvement are the development of hybrids (involving dwarfs and tall morphoforms),

which are early bearing, resistant to diseases, with an enhanced yield of copra, development of genotypes resistant to lethal yellowing disease in Latin America and Caribbean islands utilizing the resistant Malayan dwarfs from Malaysia (Been 1981), and exploitation of mutant forms of coconut ('*Makapuno*'), characterized with a jelly-like endosperm (Zuñiga 1953)—a high-value commercial product. However, developments in genetics and molecular biology (reviewed by Rajesh et al. 2018a) have enriched the genomic resources of coconut for applying multi-omics approaches (Ramesh et al. 2020a).

2.2 Coconut Genome

Coconut has a chromosome number of $2n = 32$ with an estimated genome size of 2950 million base pairs (Mbp) (Gunn et al. 2015). Flow cytometry has revealed that the average nuclear DNA content of coconut to be 5.6 ± 0.2 pg DNA/2C ($\simeq 5.4 \times 10^9$ bp). Whole-genome sequencing of a crop benefits crop improvement programs since identifying molecular markers linked to traits of economic importance and mining for novel gene(s) and alleles becomes relatively easy. Next-generation sequencing (NGS) technologies have enabled the discovery of genomic features of coconut (Alsaihati et al. 2014; Xiao et al. 2017; Lantican et al. 2019; Rajesh et al. 2020; Rajesh et al. 2021a, b, c). Among the other economically important palms, genome sequence resources are available for oil palm and date palm (Al-Mssallem et al. 2013; Dussert et al. 2013; Singh et al. 2013; Al-Dous et al. 2016). The organellar genomes (chloroplast and mitochondrial genomes) of coconut have also been made available (Huang et al. 2013; Aljohi et al. 2016; Rajesh et al. 2020; Ramesh et al. 2021b) (Table 2.1).

The genome sequence features of the Hainan tall cultivar (Xiao et al. 2017) revealed that the coconut genome harbors 28,039 protein-coding genes, relatively less than *Elaeis guineensis* (34,802) and *Phoenix dactylifera* (28,889–41,660) (Al-Mssallem et al. 2013; Singh et al. 2013; Al-Dous et al. 2016). Further, more than 70% of the coconut genome comprises transposable elements predominated by long-terminal repeat (LTR) elements (Xiao et al. 2017). Bayesian molecular clock analysis showed that coconut diverged from the oil palm around 46 million years ago. Also, the coconut genome exhibits expansion of gene families coding for Na^+/H^+ antiporters, carnitine/acylcarnitine translocases, potassium-dependent sodium-calcium exchangers, and potassium channels which are crucial in adaptation to saline environments, where the crop is predominantly grown (Xiao et al. 2017).

Application of multiple sequencing technologies such as Illumina Miseq, PacBio SMRT and Dovetail Chicago uncovered the complete genome sequence of a dwarf cultivar 'Catigan Green Dwarf' (CATD) (Lantican et al. 2019). Comparative genomic analysis of dwarf and tall coconut genome sequences identified novel 7,139 simple sequence repeats (SSRs), 58,503 single nucleotide polymorphisms (SNPs) and 13 gene-linked SSR markers (Lantican et al. 2019). This study also unraveled 34,958

Table 2.1 Salient highlights of genome sequence assemblies and organellar genomes of coconut

Sl. no.	Genotype/cultivar	Sequencing approach	Genome length	Sequence features	References
	Nuclear genomes				
1	cv. Hainan Tall	Illumina HiSeq 2000 platform	2.42 Gbp	• Scaffold length of 2.20 Gb (90.91% genome) • Over 28,000 protein-coding genes • Analysis of genes encoding cation/anion exchangers and metal transporters	Xiao et al. (2017)
2	cv. Catigan Green Dwarf	PacBio SMRT, Illumina paired-end MiSeq and Chicago sequencing	2.1 Gbp	• 35,000 gene models • 7,139 unique SSRs and ~58,000 SNP variants	Lantican et al. (2019)
3	cv. Chowghat green dwarf	Illumina HiSeq 4000 and PacBioRSII	1.93 Gbp	• ~13,000 genes • 112 nucleotide-binding site-leucine-rich repeat (NBS-LRR) loci for disease resistance • Assembly and annotation of organelle genomes	Rajesh et al. (2020)
	Organellar genomes				
4	Dwarf cultivar (chloroplast)	Illumina GAIIx	154.731 Kbp	• Encodes 130 genes and four pseudogenes • Conserved gene structure and overall organization as in palms	Huang et al. (2013)

(continued)

gene models linked to various traits along with genes underlying drought response, tolerance to biotic stress and endosperm oil synthesis.

Rajesh et al. (2020) have characterized the complete nuclear and organellar genomes of a dwarf cultivar (Chowghat Green Dwarf; CGD). It predicted that the genome harbors relatively few genes (13,707 genes encoding 11,181 proteins)

Table 2.1 (continued)

Sl. no.	Genotype/cultivar	Sequencing approach	Genome length	Sequence features	References
5	cv. Oman local tall (mitochondria)	Roche/454 GS FLX	678.65 Kbp	• Characterized to encode 72 proteins, nine pseudogenes • Chloroplast derived regions accounted for over 5% of the mitochondrial genome	Aljohi et al. (2016)
6	cv. Chowghat green dwarf (chloroplast)	Illumina HiSeq 4000 and PacBioRSII	154.628 Kbp	• Encodes 129 genes, with 84 protein-coding genes, 38 tRNAs, and two copies of four rRNAs	Rajesh et al. (2020)
7	cv. Chowghat green dwarf (mitochondria)	Illumina HiSeq 4000 and PacBioRSII	744.799 Kbp	• GC content of 41.86% • Encodes 123 genes	Rajesh et al. (2020)

because of the stringent filtering conditions followed in the analysis. The predicted genes were further validated based on transcriptome analysis of multiple coconut tissues. Interestingly, the dwarf cultivar-CGD-possesses disease resistance traits; hence, the genome sequence features would greatly help accelerate genomics assisted resistance breeding in coconut (Rajesh et al. 2020).

The genome sequence features of coconut are a valuable genetic resource for the generation of marker-saturated linkage maps, identification of novel microsatellites, tagging of quantitative trait loci (QTLs), genome-wide association studies (GWAS), and development of genomic selection (GS) models so that genomic designing of abiotic stress tolerance in coconut becomes a reality.

2.3 Coconut and Abiotic Stressors

Coconut is generally grown in coastal and island ecosystems, which are ecologically sensitive. In India, coconut is mainly cultivated in the states of Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Telangana, Maharashtra, West Bengal, Assam and Andaman and Nicobar and Lakshadweep group of islands. More than 2/3rd of coconut production in India is confined only to 20 odd districts of Southern States, thereby making the cultivation further prone to the vagaries of weather and other abiotic stresses. The United Nations Framework Convention on Climate Change

(UNFCCC) acknowledges climate change on crops and distinguishes the effect of climate variability on crops.

Rainfall, temperature, and atmospheric CO_2 concentration are the major climatic variables that severely affect the growth and development of crops. Moreover, coconut being a perennial crop, in all probability, will face abiotic stressors such as elevated CO_2 (ECO_2), elevated temperatures (ET), frequent droughts and flood situations during its lifetime. Elevated temperature (ET) conditions prevailing during crop growth severely interfere in the photosynthetic process, thereby affecting carbohydrate mobilization to the sink tissues from the source (Kumar et al. 2008; Asseng et al. 2015; Hatfield and Prueger 2015) and consequently resulting in the shortening of the growth period. ET and drought stressors during critical periods such as during inflorescence development seriously impact the nut yields of current and following three or more years (Kumar et al. 2002; Rajagopal and Kasturi Bai 2002). However, low temperatures severely damage the plant tissues to slow the process of basal metabolism affecting normal growth and development. The predictions of droughts and high rainfall conditions imply that plantation crops such as coconut will undergo multiple stresses during the same growing season (Kumar et al. 2012).

Along with the drought conditions, increased sunlight levels induce extensive damages to the cellular membranes of coconut palms due to the photo-oxidation process, consequently causing leaf scorching and severe yield decline (Kumar and Kasturi Bai 2009). On the other hand, a projected increase in atmospheric CO_2 concentration could enhance biomass accumulation in C3 crops like coconut (Ainsworth and Long 2005). It is also predicted that projected yield levels of coconut grown in India's Western coastal regions and northeastern regions could increase (Kumar and Aggarwal 2013). However, it is important to supply the necessary quantum of water and basal nutrients to harvest the benefits of enhanced biomass accumulation under ECO_2 and avoid any decline in protein accumulation due to nitrogen limiting conditions. Gomes and Prado (2007) have demonstrated that coconut palms have anatomical and biochemical characteristics, including dense stomata, dense root system, and stored sugars to tide over drought-like situations.

At the protein level, increased concentration of heat-stable protein fractions was found when coconut was subjected to ET stress, flooding or high irradiance (Kumar et al. 2007). Further, leaf epicuticular wax on its upper epidermis is an important adaptive mechanism in coconut under drought stress in cultivars and hybrids (Kurup et al. 1993; Kumar et al. 2000). Further, the effect of water deficit stress on the physiology and biochemistry of coconut productivity (Gomes et al. 2008) reveals that besides epicuticular wax, scalariform thickening in tracheids is an important attribute that defines drought tolerance in coconut (Kumar et al. 2000; Kasturi Bai et al. 2009). It was also deduced that dwarf coconuts tend to consume more water because of their relatively high stomatal frequency, little epicuticular wax and inappropriate stomatal regulation (Rajagopal et al. 1990). In contrast, tall morphoforms have physiological and biochemical adaptive mechanisms to conserve water use and improve water use efficiency (WUE) (Voleti et al. 1993).

2.4 Approaches to Investigate the Effect of Abiotic Stressors in Coconut

Considering the long term effect of abiotic stresses and its severe influence on the productive potential of coconut, multiple approaches are followed to investigate their effects. Generally, these strategies involve (a) studying the response of coconut to ECO_2 and ET, (b) phenotyping for improved water use efficiency (WUE) and drought resistance, (c) molecular approaches to decipher abiotic stress responses, and (d) simulation analysis and prediction models (Fig. 2.1).

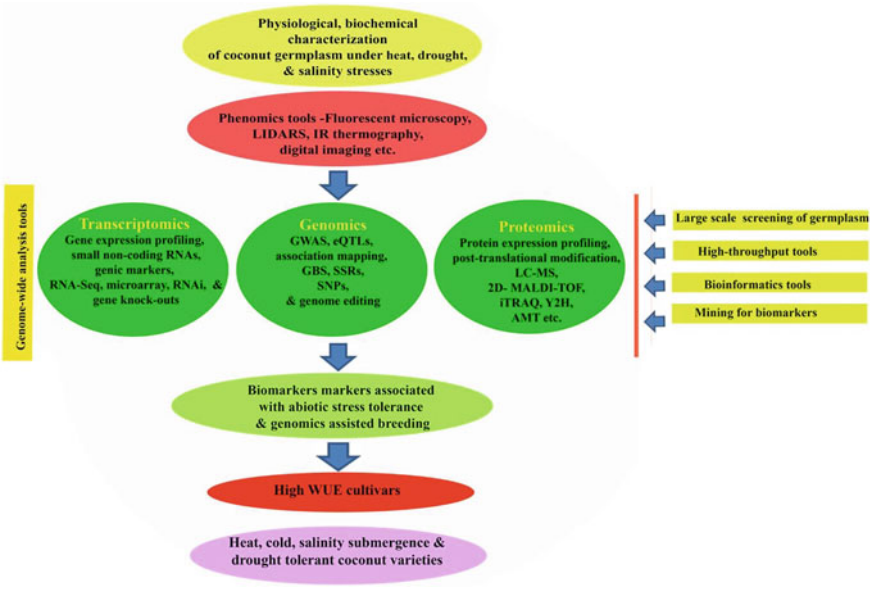

Fig. 2.1 Strategies for genomic designing of abiotic stress-tolerant coconut. [Phenotyping of coconut genotypes utilizing field screening techniques, Open Top Chamber (OTC) facilities, and phenomics modules such as fluorescent microscopy, Light Detection and Ranging (LIDAR) sensors, Infrared (IR) thermography, digital imaging along with biochemical and phenotypic markers for various abiotic stresses to identify accessions with improved resistance or tolerance. In the next stage, adoption of genome-wide analysis tools viz., RNA sequencing, gene expression analysis, identification of small and long non-coding RNAs, RNA interference (RNAi), genome-wide association studies (GWAS), expression quantitative trait loci (eQTL), genotyping by sequencing (GBS), mining of simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), complementing techniques such as liquid chromatography–mass spectrometry (LC–MS), matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), isobaric tags for relative and absolute quantification (iTRAQ), yeast-2-hybrid assays (Y2H), accurate mass and retention time (AMT) tag proteomics will delineate protein or peptide signature sequences linked to abiotic stress tolerance. The outcome of application of these techniques will lead to development of cultivars having enhanced abiotic stress tolerance including improved water use efficiency (WUE)]

Elevated CO_2 [ECO_2] and Coconut

Coconut seedlings exposed to ECO_2 (550 and 700 ppm) stresses have demonstrated enhanced growth and development owing to increased CO_2 assimilation and generation of photosynthates, ultimately improving the plant biomass. Seedlings subjected to 550 and 700 ppm of CO_2 exhibited an 8 and 25% increase in biomass, respectively, compared to that grown under 380 ppm (Hebbar et al. 2013a). Further biochemical characterization of seedlings subjected to ECO_2 showed increased photosynthates, namely sugars, reducing sugars, amino acids, and starch content. However, the reduced polyphenol content and decline in polyphenol oxidase activity in the leaves might predispose the plants to biotic stresses such as pests and pathogens (Sunoj et al. 2013).

Elevated Temperature [ET] and Coconut

Elevated temperature (ET) conditions (3 °C above 31 °C) severely reduce photosynthesis and, ultimately, photosynthates (sugars and starch) due to the reduction in leaf area growth and reduced chlorophyll content. Nevertheless, ET improves epicuticular wax deposition on the upper epidermis of leaves (Hebbar et al. 2013b). Similarly, studying the influence of ET on copra oil composition suggested that the ratio of saturated to unsaturated fatty acids increased with an increase in minimum temperature. In contrast, a decline in the ratio was observed with an increase in maximum temperature (Kumar 2005). ET caused a severe reduction in copra or nut yield; nonetheless, oil content increased (Kumar 2005).

Studies on in vitro pollen germination revealed that tall cultivars such as West Coast Tall (WCT), Federated Malay States Tall (FMST), dwarf cultivar Chowghat Orange Dwarf (COD) and hybrids [COD × WCT; MYD (Malayan Yellow Dwarf) × WCT] exhibit adaptability to elevated temperatures compared to dwarf MYD, which was least adaptable (Hebbar et al. 2018). Effect of elevated temperature on the pollen germination and identification of cardinal temperatures (T_{min}, T_{opt} and T_{max}) for the cultivars categorized them into three types (Hebbar et al. 2018).

Category 1: (high T_{opt} and low T_{min} and high T_{max})—wide adaptability (e.g. WCT, FMST, LCT, COD, hybrids)
Category 2: (relatively tolerant to T_{max})—moderately adaptable (e.g. PHOT, CCNT, GBGD, CGD and CRD)
Category 3: (high T_{min} and low T_{max})—least adaptable (MYD).

Similarly, ET stress has been identified as an important yield-limiting factor for coconut cultivation in Sri Lanka (Pathiraja et al. 2017). Screening of coconut hybrids for heat tolerance based on pollen germination revealed that Sri Lanka Green Dwarf (SLGD) × Sri Lanka Tall and Sri Lanka Brown Dwarf (SLBD) × SLGD were tolerant (Ranasinghe 2010).

Studies on the progamic phase of the tall coconut cultivar WCT and the effect of high temperature on the phase under both in vivo and in vitro conditions revealed that coconut has a long pistil, and its length was found to be 18.2 ± 4.9 mm in WCT. High temperature ($T_{max} > 33$ °C), both under in vivo and in vitro conditions, significantly reduced pollen tube growth through the pistil, suggesting its inability to reach the ovule on time to effect fertilization. High temperature also advanced nectar secretion and stigma receptivity, and the receptive stigma was dry without nectar, rendering it unappealing to insect pollinators. Thus, poor pollination and the inability of the pollen tube to reach the ovule on time to effect fertilization are the prime causes for poor nut set in the coconut variety WCT under high temperature (Hebbar et al. 2020).

Effect of Salinity Stress on Coconut

Physiological and biochemical response of coconut cultivar WCT to salinity stress revealed that seedlings could tolerate seawater substitution equivalent of 8.32 dS m^{-1} EC without affecting growth and physiological parameters. Further K^+/Na^+ ratio and tolerance to Cl^- were found to be the underlying mechanism of saltwater tolerance in coconut (Hebbar et al. 2021). Earlier, da Silva et al. (2016) identified that young 'Green Dwarf' coconut could tolerate soils with salinity levels up to electrical conductivity of 6.50 dS m^{-1}, however only when an adequate water supply was made available.

Effect of Heavy Wind Stress on Coconut

Of the weather parameters, minimum temperature had a greater influence on coconut yield than relative humidity and wind speed (Das et al. 2020). The habit features and general architecture of the coconut palms, with a relatively small trunk circumference and the tightly held upper cluster of leaves, are salient adaptation strategies to evade the damages of strong wind. Some coconut growing locations are constantly exposed to exceedingly high wind speeds, which are turning into hurricanes, cyclones in places such as Andhra Pradesh (O'Hare 2001) and Odisha (erstwhile Orissa) (Dash et al. 2002) states of the eastern coast of India, Jamaica (Been 2005), and Vanuatu (Labouisse et al. 2007). The damage due to wind could be felling/uprooting of palms or breakage of the trunk, twisting of leaf and crown etc., Recently, Subramanian et al. (2018) investigated the effect of *Gaja* cyclone on coconut plantations in the Indian state of Tamil Nadu and suggested suitable actionable strategies to revive the livelihood of farmers affected by the windstorm. It was observed that severe windstorms could result in complete uprooting of trees, trunk breakage and crown twisting, among others (Subramanian et al. 2018).

The Dwarf morphoforms of coconut were relatively susceptible to high winds than the Tall forms and hybrids; however, genotypic differences were observed (Labouisse et al. 2007). Selfed plants of Malayan Dwarf cultivar suffered heavily due to cyclonic winds than their hybrids during Hurricane Gilbert in Jamaica in 1988 (Johnston et al. 1994). The MayPan hybrids, derived by crossing Malayan Dwarf and Panama Tall, displayed more tolerance to windstorms than the Malayan Dwarf (Zizumbo et al. 2005). Tacunan Green Dwarf from the Philippines, Vanuatu Tall from Vanuatu (Labouisse et al. 2007), and other varieties from cyclone-prone areas also displayed wind tolerance due to the evolution of several adaptation traits. Traits of coconut palm such as stem height and base width/bole diameter (Johnston et al. 1994; Labouisse et al. 2007) determine its response to wind speed. Palms with thick (Zizumbo et al. 2005) and flexible trunk, tightly bound young leaves, and possessing the ability to detach old leaves (Foale 2003) during wind stress offer a high degree of tolerance to windstorms. Further, other physical traits of the trunk wood, such as its strength and elasticity, though not investigated in-depth, could contribute to resistance against the wind (Labouisse et al. 2007). Coconut genotypes originating from the South Pacific or the Philippines were resistant to the wind compared to varieties developed from Papua New Guinea (Labouisse et al. 2007).

Molecular Approaches

Application of genomics technologies including whole genome sequencing of diverse coconut cultivars (Xiao et al. 2017; Lantican et al. 2019; Rajesh et al. 2020), whole transcriptome sequencing to decipher the molecular basis of fatty acid biosynthesis (Fan et al. 2013), RNA-directed DNA methylation (Huang et al. 2014), somatic embryogenesis (Rajesh et al. 2016), diseases (Nejat et al., 2015; Rajesh et al., 2015, 2018b; Gangaraj and Rajesh, 2020) and WUE in coconut (Ramesh et al. 2020b), have yielded enormous data for practical use in the development of climate-smart coconut. Expression of protein profile in coconut cultivars has uncovered the role of heat-stable superoxide dismutase isozymes in the leaves of coconut (Kumar et al. 2014). Similarly, the application of iTRAQ-based global proteome expression profiling has revealed the role of proteins in conferring cold temperature tolerance (Yang et al. 2020).

Statistical Analysis

Peiris (2006) utilized statistical simulation models and predicted that changes in rainfall distribution during monsoon and maximum temperature are major deciding factors of coconut production in the context of climate change phenomenon. Statistical forecasting estimated that coconut production in the year 2040 would be insufficient to meet the demands even if all other external climatic determinants remain

unaltered (Peiris 2006). Further, it was also deduced that changes in climatic parameters could contribute to more than half of yield variations in coconut (Fernando et al. 2007).

Simulation Models

Simulation models (such as InfoCrop-COCONUT) are very useful tools to predict future climatic conditions and devise suitable crop husbandry approaches to counter the regional impacts of climate change and assess the vulnerability indices. Also, appropriate land use planning and yield forecasting are possible with the adoption of remote sensing and geographic information system (RS-GIS) along with crop simulation models. Simulation analysis has predicted an increase in coconut productivity of 20% in 2080 over the yield levels recorded in 2009. Further, western coastal regions of India would witness a 39% yield increase, whereas, on the eastern coast, a decline in the yield of around 31% is forecasted for the year 2080 over the yield levels of 2009 (Kumar and Aggarwal 2009). In addition, appropriate adaptation strategies and adoption of current crop management aspects would increase the productivity of the crop by 4.3% for 2030 and 6.8% for 2080 over the 2005 productivity levels (Kumar and Aggarwal 2013). In the Sri Lankan context, forecasting has predicted that annual rainfall from January to March largely determines coconut yield, and temperature and relative humidity are the major contributors to nut production (Peiris et al. 2004). In addition, the analytic hierarchy process (AHP)-based expert system was effectively used to predict the yield levels and devise suitable adaptation strategies in Sri Lanka (Pathiraja et al. 2017).

2.5 Strategies for Developing Abiotic Stress Tolerance in Coconut

Being a perennial crop, coconut necessitates the adoption of multiple strategies in developing and introgression of abiotic stress tolerance traits. The crop needs an average monthly rainfall of 150 mm for normal growth and economic nut yield. Further, a dry spell spread over 5 months could severely impede the growth of palms, consequently affecting the yield levels for the ensuing two or three years. Hence, incorporating drought tolerance or water-deficit stress tolerance traits in coconut cultivars attains great significance.

Drought Tolerance

Large scale information generated from the investigations of the physiological, biochemical process of coconut subjected to water-deficit stress or drought stress has proposed and enumerated suitable parameters such as epicuticular wax on the leaf surface, sparse stomatal density, low leaf water potential and the activities of enzymes such as like glutamate oxaloacetate transaminase (GOT) and acid phosphatase (ACP), which are linked with drought tolerance (Rajagopal et al. 1991, 2007; Chempakam et al. 1993; Kasturi Bai et al. 1996; Kumar et al. 2000). Physiological and biochemical screening have identified West Coast Tall (WCT), Federated Malay States Tall (FMST), Fiji Tall (FJT), Andaman Giant Tall (ADGT), and the hybrids, namely Laksha Ganga and Chandra Laksha, as to possessing drought tolerance trait (Rajagopal et al. 1988, 1990). Further, genetic dissection of drought-tolerant traits using line (CGD, MYD) × tester [East Coast Tall (ECT), Philippines Ordinary Tall (PHOT), Laccadive Tall (LCT), FMST] analysis revealed that traits such as transpiration rate, lipid peroxidation, water potential and photosynthetic rates exhibited both the additive and non-additive gene actions (Rajagopal et al. 2007). The high specific combining ability for the trait, transpiration rate suggested the phenomenon of heterosis for this trait. However, heterosis breeding could be exploited for yield improvement using the parameter photosynthetic rate since this trait was governed by non-additive gene action. Hence, the identified genotypes and the physiological/biochemical parameters are being utilized in breeding and evolving high yielding and drought-tolerant hybrids in ICAR-CPCRI, Kasaragod (Table 2.2).

Table 2.2 List of prominent drought-tolerant coconut varieties developed in India

Variety	Characteristic features	Institution
Chandra Kalpa	Drought-tolerant, high oil (72%)	ICAR-CPCRI, India
Kalpa Mitra	Drought-tolerant, high nut and oil yield	ICAR-CPCRI, India
Kalpa Dhenu	Drought-tolerant, high nut and oil yield	ICAR-CPCRI, India
Kalpatharu	Drought-tolerant, high yield, ball copra	AICRP on Palms (AICRPP), ICAR-CPCRI, India
VPM-3	Drought-tolerant, high yield	Tamil Nadu Agricultural University (TNAU), India
Kalpa Ratna	Tolerant to moisture stress, multipurpose variety suitable for tender nut, copra/oil, inflorescence sap (*neera*) production	ICAR-CPCRI, India

Climate Resilience

In the context of climate change, it was predicted that the increase in greenhouse gas concentrations (from 380 ppm to between 500 and 970 ppm) would cause a rise in global temperature increase between 1 and 5.5 °C for 2100 (Pachauri and Reisinger 2007). As already stated, coconut is a C3 crop; hence an increase in atmospheric CO_2 concentration would increase the biomass, however with a concomitant demand for plant nutrients (Cavagnaro et al. 2011). Growth and development of coconut seedlings in the open-top chamber (OTC) under the combined treatments of ECO_2 and ET demonstrate that seedlings grown under ECO_2 accumulate significantly high biomass of 1.13 and 1.98 kg seedling^{-1} under 550 and 700 ppm CO_2 compared to 1.1 kg seedling^{-1} under ambient conditions (Hebbar et al. 2013b). It was deduced that physiological parameters such as stomatal conductance and transpiration rate remain reduced under ECO_2 without compromising the rate of photosynthesis, thereby improving the WUE of the seedlings.

Screening for Cold Tolerance

Coconut is a tropical palm; hence any decline in ambient or atmospheric temperature could cause severe cold injury, and there is a need to develop cold-tolerant genotypes. Also, the atmospheric temperature below 13 °C severely affects the flowering and fruit set besides causing damage to spear leaf, drying of leaf and wrinkled endosperms (Mao 1986). Screening studies of coconut accessions for cold stress tolerance have identified that Hainan Tall of China and Kamrup Tall of India possess cold tolerance (Mao and Lai 1993; Chowdhury et al. 2001).

Exploitation of Genetic Resources—A Cornerstone for Modern Plant Breeding

Screening of coconut germplasm for tolerance towards various abiotic stresses and identification of genetic sources for tolerance to ET stress, enhanced pollen viability during high-temperature stress, nut retention trait at high temperature, high WUE, salinity tolerance are undertaken to develop stress-tolerant genotypes of coconut (Hebbar et al. 2016, 2018, 2021). Extensive use of drought-tolerant genotypes in crop improvement programs have yielded many varieties (Chandra Kalpa, Kalpatharu, KeraKeralam, Kalpa Mitra, Kalpa Dhenu, KeraSankara, and Chandra Laksha) having enhanced drought tolerance trait (Kasturi Bai et al. 2009).

To develop drought-tolerant coconut varieties, it is imperative to screen the accessions for the WUE trait, governed by efficient root systems to absorb water and effective control of stomatal movement in regulating water loss (Hebbar et al. 2016).

Extensive screening at ICAR-CPCRI has identified that tall genotypes Kalpadhenu, FMST and Kalpatharu show appreciable WUE owing to the effective root system, whereas dwarfs also exhibit improved WUE due to stomatal regulation. In addition, in vitro pollen germination is an important criterion for heat stress tolerance since pollination is a crucial stage that is susceptible to ET conditions. In vitro screening of pollen germination under ET conditions have identified that talls such as WCT, LCT, FMST and dwarf COD and hybrids are adaptable, and the dwarf MYD was found to be least adaptable to high-temperature conditions (Hebbar et al. 2018).

Genomic Approaches for Incorporating Abiotic Stress Tolerance in Coconut

Applying conventional breeding and screening approaches to complex traits such as drought tolerance, resistance to ET, and improved WUE is a cumbersome process in a highly heterogeneous, heterozygous, cross-breeding population like coconut. Hence, to circumvent these issues, it is imperative to adopt genomic approaches to develop coconut with abiotic stress tolerance. Also, next-generation breeding greatly relies on the large plant breeding populations and germplasm collections, assisted by high-throughput technologies, big data analytics, followed by biotechnological and molecular breeding strategies.

DNA Markers and QTL Mapping

In coconut, DNA-based molecular markers such as RAPD (Ashburner et al. 1997), RFLP (Lebrun et al. 1998), AFLP (Perera et al. 1998) and SSRs (Perera et al. 2003) were utilized for dissecting the genetic diversity, association analysis (Geethanjali et al. 2018) and marker-assisted breeding. Molecular marker-trait linkage and mapping of QTLs are integral components of genomics aided breeding. Application of DNA-based molecular markers in QTL mapping of traits of economic importance such as earliness in flowering (Herran et al. 2000), economic nut yield (Lebrun et al. 2001) and fruit quality features (Baudouin et al. 2006) and leaf epidermis wax composition (Riedel et al. 2009) are worth mentioning. In coconut, various molecular markers, namely AFLP, ISSR, ISTR, RAPD and SSR, were used to study QTLs governing traits such as germination, yield and yield attributing traits and epicuticular wax content for improved abiotic stress tolerance coconut (Table 2.3).

Genome-Wide Association Studies (GWAS) and Genomic Selection

GWAS in coconut is scarce, excluding a report by Geethanjali et al. (2018) wherein SSR markers linked to fruit component traits have been obtained. It is pertinent to

Table 2.3 Application of molecular assisted breeding in mining QTLs of importance in coconut to develop abiotic stress tolerance

Sl. no	Mapping population	DNA markers	QTL characteristic features	References
1	Malayan Yellow Dwarf (MYD) × Laguna Tall (LAG)	ISTR	–	Rohde et al. (1999)
2	Laguna Tall × Malayan Yellow Dwarf (MYD)	AFLP, ISSR, ISTR and RAPD	Six QTLs linked to precocious germination, early flowering and yield	Herran et al. (2000)
3	Cameroon Red Dwarf (CRD) × Rennell Island Tall (RIT)	AFLP and SSR]	Nine QTLs associated with yield and yield attributing traits, namely bunch and nut number	Lebrun et al. (2001)
4	Cameroon Red Dwarf (CRD) × Rennell Island Tall (RIT)	Oil palm derived AFLP, SSRs and coconut SSRs	48 QTLs linked with fruit component traits	Baudouin et al. (2006)
5	African Tall (EAT) × Rennell Island Tall (RIT)	241 AFLP, 64 SSRs and 22 SSRs from oil palm	46 QTLs associated with epicuticular wax content	Riedel et al. (2009)
6	79 genotypes across the world	SSRs	Linkage disequilibrium-based mapping identified SSR locus CnCir73 linked to fruit component traits	Geethanjali et al. (2018)

develop a highly saturated linkage map in coconut so that it aids in the improvement of breeding efficiency (Rivera et al. 1999). A robust linkage map is being developed using a core set of SNP and SSR markers in coconut. Though the development and validation of molecular markers have potential utility in molecular breeding are cumbersome, NGS technologies have offered various avenues to rapidly develop markers at a relatively low cost. In this framework, genotype-by-sequencing (GBS) allows for genome-wide discovery of novel markers and genotyping utilizing NGS technologies. Concurrent discovery of markers and genotyping is possible using the GBS technique as the genomic complexity is reduced using restriction enzymes and DNA barcoded adapters (Chung et al. 2017).

Muñoz-Peréz et al. (2020) combined the population genetics and phenotyping analysis while exploring the genetic diversity of coconut genotypes derived from the Atlantic and Pacific coasts of Colombia. GBS-derived SNPs from these genotypes were analyzed, further corroborating the independent origin of coconut cultivation in Pacific and Indo-Atlantic Ocean basins (Gunn et al. 2011). Molecular evolutionary genomic analysis suggests that Atlantic talls diversified from the dwarfs and talls of

the Pacific almost 5400 years ago. The coconut genetic loci governing domestication traits, including reproduction and defence, have been characterized. Thus, the GBS approach helped in mining high-quality SNPs and in resolving questions related to population genetics in coconut (Muñoz-Peréz et al. 2020).

GBS analysis of 38 Thailand coconut accessions helped in mining a total of 22,748 loci having SSR motifs, 2452 polymorphic loci and 315 SSR primer pairs (Riangwong et al. 2020). Further, 74 SSR primers were used in genotyping exercise in 40 coconut accessions comprising tall and dwarf forms identifying two and nine alleles per locus in talls, whereas one to four alleles were identified in dwarfs. Thus, the GBS approach offers a great potential to mine more molecular markers with immense utility in coconut breeding (Riangwong et al. 2020).

Recently, Rajesh et al. (2021c) have utilized the GBS approach to genotype an association mapping panel of 96 coconut palms, comprising 16 accessions from geographically different locations. The 10,835 high-quality SNPs identified were employed to assess genetic diversity, population structure, and linkage disequilibrium (LD). The genetic diversity analysis divulged a high level of variation in the 96 genotypes. Unweighted neighbor-joining phylogenetic tree and Bayesian-based model population structure classified the palms into four main clusters. A relatively rapid LD decay with a short-range (9 kb) was detected. These results offer enormous opportunities for genome-wide association studies and genomic selection (GS) implementation to enhance genetic gains in coconut.

Transcriptomic Approaches for Abiotic Stress Tolerance in Coconut

Whole transcriptome sequencing using NGS approaches followed by sequence assembly is a valuable technique for investigating the global gene expression profile and identifying genic molecular markers associated with a trait. In coconut, Fan et al. (2013) applied transcriptome sequencing technique to decipher the fatty acid biosynthesis process. It identified 347 unigenes linked to fatty acid biosynthesis and a corroborative evidence for the expression of fatty acyl-ACP thioesterase in the accumulation of lauric acid in coconut. It was followed by Huang et al. (2014), who investigated the molecular factors and genetic elements associated with the RNA-directed DNA methylation process in the seeds and leaves of dwarf coconut. It has enumerated a list of highly expressed genes that could be exploited for coconut improvement using tissue-specific promoters. The transcriptomic response of coconut seedlings with contrasting WUE traits was documented by Ramesh et al. (2020b). Two-year-old coconut seedlings of Kalpa Sree and Kalpatharu were subjected to soil water-deficit regimes (25% of available soil moisture and control) and were investigated utilizing Illumina paired-end RNA-Seq. In total, ~7300 differentially expressed genes have been identified between the seedlings under water-deficit stress and control. Comparative analysis of stressed and control Kalpa Sree seedlings showed that 2388 transcripts are significantly upregulated, and 1278 are significantly downregulated, whereas Kalpatharu showed upregulation and down-regulation of 2868 and 778 transcripts, respectively. Kalpasree leaf transcriptome showed significant upregulation

of phloem protein 2-like A1-like, WRKY transcription factor 40 isoform X1 and down-regulation of glycerol-3-phosphate acyltransferase three transcripts. Upregulation of transcripts encoding polyamine oxidase, arabinose 5-phosphate isomerase, and down-regulation of aquaporin PIP1-2 transcript was documented in Kalpatharu leaves. Besides, long noncoding RNAs and genic SSRs were also identified to further enrich the genomic resources of coconut (Ramesh et al. 2020b).

Differential display analysis was performed to delineate the gene expression profile of coconut plantlets subjected to moisture stress in 20 and 30% PEG treated plantlets (Bobby Paul et al. 2010). RNA expression profiles were generated using 75 primer combinations in six different RNA samples (two from control; two from 20% PEG (5 and 10 days post-treatment); two from 30% PEG (5 and 10 days post-treatment). It revealed 129 differential amplicons between unstressed and PEG induced water-stressed plantlets. Isolation and characterization of differentially expressed transcripts identified a total of 114 genic fragments, which were cloned, and sequence information was obtained. Classification of differentially expressed genes based on DNA sequence features divulged that hierarchies of the grouped transcripts are: genes involved in abiotic stress regulation (22%), metabolic enzymes (20%), DNA binding domain (11%), and transcription factors (6%) among others (Bobby Paul et al. 2010). This was the first report of gene expressional changes in coconut during induced water stress. Also, the genes uncovered in this study are good candidate markers for use in molecular breeding for water-deficit stress tolerance in coconut (Bobby Paul et al. 2010).

Auxin response factors (ARFs) are a distinct class of plant transcription factors (TFs) that modulate the molecular response of plants to various environmental stresses. Genome-wide exploration of ARFs of coconut in CGD cultivar identified 20 genes (*CnARF*s) (Santhi et al. 2018, 2021). Gene expression analysis of mature zygotic embryo-specific ARFs subjected to osmotic and elevated temperature stresses delineated the signature transcriptional response of ARFs to specific external stressors. Although the majority of *CnARF*s of zygotic embryos exhibited down-regulation following PEG 6000, NaCl and mannitol treatments, *CnARF*4 was upregulated during osmotic stress. Also, *CnARF6*, *CnARF12*, *CnARF4* and *CnARF10* were upregulated at some time points of osmotic treatments suggesting their intricate role in stress management. In contrast, most of the CnARFs were induced following temperature stresses, as *CnARF9* was found to be upregulated following heat and cold stresses. Further expression of *CnARF4* and *CnARF6* were enhanced after 4–12 h after treatment (HAT) when subjected to high-temperature treatment, whereas *CnARF13* and *CnARF10* were upregulated only at 12 HAT. This study has laid a strong foundation for identifying functional marker(s) linked to abiotic stress tolerance, which would greatly aid in developing stress-tolerant coconut cultivars (Santhi et al. 2018, 2021).

Proteomic Approaches to Develop Abiotic Stress Tolerance in Coconut

Global proteomic expression profile in two coconut genotypes Hainan Tall, BenDi (BD) and Aromatic coconut, Xiang Shui (XS), when subjected to cold stress (8 °C for 2 and 5 days) was investigated utilizing the iTRAQ technique (Yang et al. 2020). Imposition of cold stress (8 °C) for 2 days evinced perturbations of protein expression as 193 upregulated, and 134 downregulated proteins in BD was recorded. In contrast, in the genotype XS, 140 and 155 proteins were found to be up- and downregulated, respectively (Yang et al. 2020). Similarly, an extension of cold stress for 5 days have caused a differential response in the coconut genotypes since the cultivar BD exhibited abundant upregulated proteins compared to cultivar XS (Yang et al. 2020). Further classification and characterization of differentially expressed protein during cold stress in both the coconut genotypes suggest that proteomic response to cold stress involve multiple stress responses, including abiotic, biotic and oxidative stresses implying the cross-talk at the molecular level (Yang et al. 2020). Moreover, cold stress tolerance in BD (Hainan Tall) cultivar could be due to the accumulation of select proteins involved in ROS scavenging. Furthermore, post-translational modification and protein turnover in tiding over the cold stress condition could not be overruled in coconut (Yang et al. 2020).

2.6 Way Forward and Concluding Remarks

A whitepaper prepared by Food and Agricultural Organization (FAO) in 2009 suggests the concept of climate-smart agriculture. A pre-requisite for climate-smart crop production techniques is the preservation and conservation of genetic resources. Hence, germplasm characterization, selection, and utilization warrant a novel perspective approach with the adoption of crop genomics and digital biotechnology, where the emphasis is on extracting constructive information from big data. In this context, large scale breeding population, data from low cost, high-throughput sequencing technologies, bioinformatics tools, and application of molecular breeding approaches form the cornerstones of modern breeding to develop abiotic stress tolerant coconut genotypes.

Furthermore, coconut breeding lacks the adoption of recent molecular breeding approaches such as genome-wide association analysis, the development of GS models, and rapid exploration of QTLs. Though GBS approaches are adopted to complement the availability of relatively high-quality whole-genome sequences of coconut genotypes, there is a need to generate highly marker-saturated QTL maps. In this context, diverse mapping populations such as the multi-parent advanced generation intercross (MAGIC) population and the nested association mapping (NAM) population are worth exploring to saturate the QTL maps and to usher in an era of QTLomics-backed varietal development programs.

Above all, improved comprehension of the physiological, biochemical, genetic and molecular basis of heat, water-deficit, salinity and ECO_2 stresses in coconut is

pertinent to integrate the outcomes of basic science with that of genomics assisted breeding for tolerant crops. In this context, the large scale application of transcriptomics, proteomics to dissect the stress responses in coconut would yield immense knowledge for genetic manipulation and genomics-aided breeding.

References

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165(2):351–372

Al-Dous EK, George B, Al-Mahmoud ME, Al-Jaber MY, Wang H, Salameh YM, Al-Azwani EK, Chaluvadi S, Pontaroli AC, DeBarry J, Arondel V (2016) *De novo* genome sequencing and comparative genomics of date palm (*Phoenix dactylifera*). Nat Biotechnol 29:521–527

Aljohi HA, Liu W, Lin Q, Zhao Y, Zeng J, Alamer A, Alanazi IO, Alawad AO, Al-Sadi AM, Hu S, Yu J (2016) Complete sequence and analysis of coconut palm (*Cocos nucifera*) mitochondrial genome. PLoS One 11(10):e0163990

Al-Mssallem IS, Hu S, Zhang X, Lin Q, Liu W, Tan J, Yu X, Liu J, Pan L, Zhang T, Yin Y (2013) Genome sequence of the date palm *Phoenix dactylifera* L. Nat Commun 4:2274

Alsaihati B, Liu W, Lin Q, AlMssallem IS (2014) Coconut genome de novo sequencing. In: Plant and animal genome conference XXII, San Diego, California, USA, PO46

Arunachalam V, Rajesh MK (2017) Coconut genetic diversity, conservation and utilization. In: Ahuja MR, Jain SM (eds) Biodiversity and conservation of woody plants. Sustainable development and biodiversity, vol 17. Springer, Cham, pp 3–36

Arunachalam V, Rajesh MK (2008) Breeding of coconut palm. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 3(053):1–12

Ashburner GR, Thompson WK, Halloran GM (1997) RAPD analysis of south pacific coconut palm populations. Crop Sci 37:992–997

Asseng S, Ewert F, Martre P, Rötter RP, Lobell DB, Cammarano D, Kimball BA, Ottman MJ, Wall GW, White JW, Reynolds MP (2015) Rising temperatures reduce global wheat production. Nat Clim Change 5(2):143

Baudouin L, Lebrun P, Konan JL, Ritter E, Berger A, Billotte N (2006) QTL analysis of fruit components in the progeny of a Rennell Island tall coconut (*Cocos nucifera* L.) individual. Theor Appl Genet 112:258–268

Been BO (1981) Observations on field resistance to lethal yellowing in coconut varieties and hybrids in Jamaica. Oléagineux 36:9–11

Been BO (2005) Status of coconut genetic resources research in Jamaica. In: Batugal P, Ramanatha Rao V, Oliver J (eds) Coconut genetic resources. International Plant Genetic Resources Institute, United States, pp 715–719

Bobby P, Rajesh MK, Kiran AG, Thomas GV, Naresh Kumar S (2010) Differential expression of genes regulated in response to water stress in coconut. In: Thomas GV, Krishnakumar V, Jerard BA (eds) Improving productivity and profitability in coconut farming. Proceedings of international conference on coconut biodiversity for prosperity, pp 116–122

Burton J (2019) The world leaders in coconut production. World Atlas. https://www.worldatlas.com/articles/the-world-leaders-in-coconut-production.html. Accessed 2 Nov 2019

Buschena DE, Perloff JM (1991) The creation of dominant firm market power in the coconut oil export market. Am J Agric Econ 73(4):1000–1008

Cavagnaro TR, Gleadow RM, Miller RE (2011) Plant nutrient acquisition and utilisation in a high carbon dioxide world. Funct Plant Biol 38(2):87–96

Chempakam B, Kasturi Bai KV, Rajagopal V (1993) Lipid peroxidation and associated enzyme activities in relation to screening for drought tolerance in coconut (*Cocos nucifera* L.). Plant Physiol Biochem 20:5–10

Child R (1974) Coconut, 2nd edn. Longman, London

Chowdhury D, Nath JC, Mohan NK (2001) 'Kamrupa'—a newly released coconut variety by Assam Agricultural University. Indian Coconut J 31:12–13

Chung YS, Choi SC, Jun TH, Kim C (2017) Genotyping-by-sequencing: a promising tool for plant genetics research and breeding. Hortic Environ Biotechnol 58:425–431

Das B, Nair B, Arunachalam V, Reddy KV, Venkatesh P, Chakraborty D, Desai S (2020) Comparative evaluation of linear and nonlinear weather-based models for coconut yield prediction in the west coast of India. Int J Biometeorol 64(7):1111–1123

Dash DK, Ray DP, Khan HH (2002) Extent of damage to coconut palms caused by super cyclone in Orissa and pattern of their recovery. CORD (Coconut Res Dev) 18(01):34

da Silva AR, Bezerra FM, Lacerda CFD, Miranda RDS, Marques EC, Gomes-Filho E (2016) Organic solutes in coconut palm seedlings under water and salt stresses. Revista Brasileira de Engenharia Agrícola e Ambiental 20:1002–1007

Dussert S, Guerin C, Andersson M, Joët T, Tranbarger TJ, Pizot M, Sarah G, Omore A, Durand-Gasselin T, Morcillo F (2013) Comparative transcriptome analysis of three oil palm fruit and seed tissues that differ in oil content and fatty acid composition. Plant Physiol 162(3):1337–1358

Fan H, Xiao Y, Yang Y, XiaW MAS, Xia Z, Qiao F, Zhao S, Tang H (2013) RNA-Seq analysis of *Cocos nucifera*: transcriptome sequencing and *de novo* assembly for subsequent functional genomics approaches. PLoS One 8:59997

Fernando MTN, Zubair LM, Peiris TSG, Ranasinghe CS, Ratnasiri J (2007) Economic value of climate variability impacts on coconut production in Sri Lanka. Retrieved from the AIACC Project Office, International START Secretariat, Washington, DC. AIACC Working Paper No. 45, March 2007

Foale M (2003) Coconut odyssey: the bounteous possibilities of the tree of life. Australian Centre for International Agricultural Research

Gangaraj KP, Rajesh MK (2020) Dataset of dual RNA-sequencing of *Phytophthora palmivora* infecting coconut (*Cocos nucifera* L.). Data Brief 30:105455. https://doi.org/10.1016/j.dib.2020.105455

Geethanjali S, Anitha Rukmani J, Rajakumar D, Kadirvel P, Viswanathan PL (2018) Genetic diversity, population structure and association analysis in coconut (*Cocos nucifera* L.) germplasm using SSR markers. Plant Genet Resour Charact Util 16:156–168

Gomes FP, Oliva MA, Mielke MS, de Almeida AA, Leite HG, Aquino LA (2008) Photosynthetic limitations in leaves of young Brazilian Green Dwarf coconut (*Cocos nucifera* L. 'nana') palm under well-watered conditions or recovering from drought stress. Environ Exp Bot 62(3):195–204

Gomes FP, Prado CH (2007) Ecophysiology of coconut palm underwater stress. Braz J Plant Physiol 19(4):377–391

Gunn BF, Baudouin L, Beulé T, Ilbert P, Duperray C, Crisp M, Issali A, Konan JL, Rival A (2015) Ploidy and domestication are associated with genome size variation in Palms. Am J Bot 102(10):1625–1633

Gunn BF, Baudouin L, Olsen KM (2011) Independent origins of cultivated coconut (*Cocos nucifera* L.) in the Old World tropics. PLoS One 6(6):e21143. https://doi.org/10.1371/journal.pone.0021143

Hatfield JL, Prueger JH (2015) Temperature extremes: effect on plant growth and development. Weather Clim Extrem 10:4–10

Hebbar KB, Balasimha D, Thomas GV (2013a) Plantation crops response to climate change: coconut perspective. In: Singh HCP, Rao NKS, Shivashankar KS (eds) Climate-resilient horticulture: adaptation and mitigation strategies. Springer, India, pp 177–187

Hebbar KB, Sheena TL, ShwethaKumari K, Padmanabhan S, Balasimha D, Berwal MK, Thomas GV (2013b) Response of coconut seedlings to elevated CO_2 and high temperature in drought and high nutrient conditions. J Plantain Crops 41:118–122

Hebbar KB, Berwal MK, Chaturvedi VK (2016) Plantation crops: climatic risks and adaptation strategies. Indian J Plant Physiol 21(4):428–436

Hebbar KB, Rose HM, Nair AR, Kannan S, Niral V, Arivalagan M, Gupta A, Samsudeen K, Chandran KP, Chowdappa P, Prasad PV (2018) Differences in *in vitro* pollen germination and pollen tube growth of coconut (*Cocos nucifera* L.) cultivars in response to high temperature stress. Environ Exp Bot 153:35–44

Hebbar KB, Neethu P, Sukumar PA, Sujithra M, Santhosh A, Ramesh SV, Niral V, Hareesh GS, Nameer PO, Prasad PVV (2020) Understanding physiology and impacts of high temperature stress on the progamic phase of coconut (*Cocos nucifera* L.). Plants 9(12):1651. https://doi.org/10.3390/plants9121651

Hebbar KB, Santhosh A, Sukumar AP, Neethu P, Ramesh SV, Selvamani V (2021) Effect of seawater substitution on growth, physiological and biochemical processes of coconut (*Cocos nucifera* L.) seedlings—a hydroponic study. Sci Hortic 280:109935

Herran A, Estioko L, Becker D, Rodriguez MJB, Rhode W, Ritter E (2000) Linkage mapping and QTL analysis in coconut (*Cocos nucifera* L.). Theor Appl Genet 101:292–300

Huang YY, Matzke AJ, Matzke M (2013) Complete sequence and comparative analysis of the chloroplast genome of coconut palm (*Cocos nucifera*). PLoS One 8(8):e74736

Huang YY, Lee CP, Fu JL, Chang BC, Matzke AJ, Matzke M (2014) De novo transcriptome sequence assembly from coconut leaves and seeds with a focus on factors involved in RNA-directed DNA methylation. G3 Genes Genomes Genet 4(11):2147–2157

Johnston CF, Fielding WJ, Been B (1994) Hurricane damage to different coconut varieties. Trop Agric (Trinidad) 71(3):239–242

Kasturi Bai KV, Rajagopal V, Prabha CD, Ratnambal MJ, George MV (1996) Evaluation of coconut cultivars and hybrids for dry matter production. J Plantain Crops 24:23–28

Kasturi Bai KV, Naresh Kumar S, Rajagopal V (2009) Abiotic stress tolerance in coconut. CPCRI, Kasaragod, India

Kumar SN (2005) Variability in content and composition of fatty acids in coconut oil due to genetic and environmental factors. AP Cess fund project. Final report submitted to ICAR, New Delhi

Kumar SN, Aggarwal PK (2009) Impact of climate change on coconut plantations. In: Aggarwal PK (ed) Climate change and Indian agriculture. Case studies from network project on climate change. ICAR Publication, New Delhi, India, pp 24–27

Kumar SN, Aggarwal PK (2013) Climate change and coconut plantations in India: impacts and potential adaptation gains. Agric Syst 117:45–54

Kumar SN, Kasturi Bai KV (2009) Photosynthetic characters in different shapes of coconut canopy under irrigated and rainfed conditions. Indian J Plant Physiol 14(3):215–223

Kumar SN, Bai KV, George J, Balakrishnan A, Thomas ST (2007) Stress responsive proteins in coconut seedlings subjected to water, high-light, flooding and high-temperature stresses. Indian J Hortic 64(4):373–380

Kumar SN, Bai KK, Rajagopal V, Aggarwal PK (2008) Simulating coconut growth, development and yield with the InfoCrop-coconut model. Tree Physiol 28(7):1049–1058

Kumar SN, Singh AK, Rao VUM, Aggarwal PK, Venkateswarulu B (2012) Climate change and Indian agriculture: salient achievements of ICAR network project. IARI Publication, New Delhi, India, p 32

Kumar SN, Rajagopal V, Karun A (2000) Leaflet anatomical adaptations in coconut cultivars for drought tolerance. Recent advances in plantation crops research. CPCRI Contribution, Kasaragod, India, pp 225–229

Kumar SN, Rajagopal V, Siju Thomas T, Vinu K, Cherian M, Hanumanthappa M, Anil Kumar B, Srinivasulu B, Nagvekar DD (2002) Identification and characterization of *in situ* drought tolerant coconut palms in farmers' fields in different agro-climatic zones. In: Sreedharan K, Vinod Kumar PK, JayaramBasavaraj MC (eds) Proceedings of PLACROSYM XV, Kerala, India

Kumar M, Padmanabhan S, Hebbar KB (2014) Superoxide dismutase isozymes and their heat stability in coconut (*Cocos nucifera* L.) leaves. Ann Biol 30(4):593–597

Kurup VVGK, Voleti SR, Rajagopal V (1993) Influence of weather variables on the content and composition of leaf surface wax in coconut. J Plantain Crops 21(2):71–80

Labouisse JP, Sileye T, Hamelin C (2007) New observations on the resistance of coconut cultivars to tropical cyclones in Vanuatu. CORD 23(2):28–38

Lantican DV, Strickler SR, Canama AO, Gardoce RR, Mueller LA, Galvez HF (2019) *De novo* genome sequence assembly of dwarf coconut (*Cocos nucifera* L. 'Catigan Green Dwarf') provides insights into genomic variation between coconut types and related palm species. G3 Genes Genomes Genet 9:2377–2393. https://doi.org/10.1534/g3.119.400215

Lebrun P, N'cho YP, Seguin M, Grivet L, Baudouin L (1998) Genetic diversity in coconut (*Cocos nucifera* L.) revealed by restriction fragment length polymorphism (RFLP) markers. Euphytica 101:103–108

Lebrun P, Baudouin L, Bourdeix R, Konan JL, Barker JH, Aldam C, Herran A, Ritter E (2001) Construction of a linkage map of the Rennell Island Tall coconut type (*Cocos nucifera* L.) and QTL analysis for yield characters. Genome 44:962–970

Mao Z (1986) An investigation on meteorological indicers for coconut cultivation in China. Oleagineux 41:119–128

Mao Z, Lai Y (1993) The coconut germplasm of Hainan Island, China. Plant Genet Resour Newsl 91(92):53–57

Muñoz-Pérez JM, Cañas GP, López L, Arias T (2020) Genome-wide diversity of coconut from northern South America uncovers genotypes present in Colombia and strong population structure. bioRxiv 1:825398

Murray DV (1977) Coconut palm. In: Alvim TA, Kozlowski TT (eds) Ecophysiology of tropical crops. Academic Press, New York, pp 1–27

Nair RV, Jerard BA, Thomas RJ (2016) Coconut breeding in India. In: Al-Khayri JM, Jain SM, Johnson DV (eds) Advances in plant breeding strategies: agronomic, abiotic and biotic stress traits. Springer International Publishing, Switzerland, pp 257–279

Nejat N, Cahill DM, Vadamalai G, Ziemann M, Rookes J, Naderali N (2015) Transcriptomics-based analysis using RNA-Seq of the coconut (*Cocos nucifera*) leaf in response to yellow decline phytoplasma infection. Mol Genet Genom 290(5):1899–1910

O'Hare G (2001) Hurricane 07B in the Godavari Delta, Andhra Pradesh, India: vulnerability, mitigation and the spatial impact. Geogr J 167(1):23–38

Pachauri RK, Reisinger A (2007) IPCC fourth assessment report. IPCC, Geneva

Pathiraja E, Griffith G, Farquharson B, Faggian R (2017) The Economic cost of climate change and the benefits from investments in adaptation options for Sri Lankan coconut value chains. In: International European Forum, 13–17 February 2017, vol 2017, no. 1012-2017-712. Innsbruck-Igls, Austria, pp 460–485

Peiris T, Wijeratne M, Ranasinghe C, Aanadacumaraswamy A, Fernando M, Jayakody A, Ratnasiri J (2004) Impact of climate change on coconut and tea industry in Sri Lanka. Paper presented at the 2nd AIACC regional workshop for Asia and the Pacific, Manila, the Philippines

Peiris TSG (2006) Impact of climate change on coconut industry in Sri Lanka. Paper presented at third international conference on climate impact and assessment (TICCIA), Cairns, Australia

Perera L, Russell JR, Provan J, McNicol JW, Powell W (1998) Evaluating genetic relationships between indigenous coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. Theor Appl Genet 96:545–550

Perera L, Russell JR, Provan J, Powell W (2003) Studying genetic relationships among coconut varieties/populations using microsatellite markers. Euphytica 132:121–128

Rajagopal V, Kasturi Bai KV (2002) Drought tolerance mechanism in coconut. Burot Bull 17:21–22

Rajagopal V, Kasturi Bai KV, Voleti SR (1990) Screening of coconut genotypes for drought tolerance. Oleagineux 45:215–223

Rajagopal V, Kasturibai K, Shivashankar S (1991) Physiological and Biochemical bases of Coconut Production. CORD 7(02):34

Rajagopal V, Bai KV, Kumar SN, Niral V (2007) Genetic analysis of drought responsive physiological characters in coconut. Indian J Hort 64(2):181–189

Rajagopal V, Voleti SR, Kasturi Bai KV, Shivashankar S (1988) Physiological and biochemical criteria for breeding for drought tolerance in coconut.

Rajesh MK, Rachana KE, Naganeeswaran SA, Shafeeq R, Thomas RJ, Shareefa M, Merin B, Anitha K (2015) Identification of expressed resistance gene analog sequences in coconut leaf transcriptome and their evolutionary analysis. Turk J Agric For 39(3):489–502

Rajesh MK, Rachana KE, Kulkarni K, Sahu BB, Thomas RJ, Karun A (2018b) Comparative transcriptome profiling of healthy and diseased Chowghat Green Dwarf coconut palms from root (wilt) disease hot spots. Eur J Plant Pathol 151(1):173–193

Rajesh MK, Ramesh SV, Perera L, Kole C (2021a) The coconut genome. Springer International Publishing, Cham, p 207p

Rajesh MK, Ramesh SV, Karun A, Chowdappa P (2021b) Genome sequencing, transcriptomics, proteomics and metabolomics. In: Rajesh MK, Ramesh SV, Perera L, Kole C (eds) The coconut genome. Springer International Publishing, Cham, pp 119–132

Rajesh MK, Gangurde SS, Pandey MK, Niral V, Sudha R, Jerard BA, Kadke GN, Sabana AA, Muralikrishna KS, Samsudeen K, Karun A, Keshava Prasad TS (2021c) Insights on genetic diversity, population structure, and linkage disequilibrium in globally diverse coconut accessions using genotyping-by-sequencing. OMICS 25(12):796–809

Rajesh MK, FayasTP, Naganeeswaran S, Rachana KE, Bhavyashree U, Sajini KK, Karun A (2016) De novo assembly and characterization of global transcriptome of coconut palm (*Cocos nucifera* L.) embryogenic calli using Illumina paired-end sequencing. Protoplasma 253(3):913–928.

Rajesh MK, Karun A, Parthasarathy VA (2018a) Coconut biotechnology. In: Nampoothiri KUK, Krishnakumar V, Thampan P, Nair MA (eds) The coconut palm (*Cocos nucifera* L.)—research and development perspectives. Springer, Singapore, pp 191–226.

Rajesh MK, Chowdappa P, Behera SK, Kasaragod S, Gangaraj KP, Kotimoole CN, Nekrakalaya B, Mohanty V, Sampgod RB, Banerjee G, Das AJ, Niral V, Karun A, Mahato AK, Gaikwad K, Singh NK, Keshava Prasad TS (2020) Assembly and annotation of the nuclear and organellar genomes of a dwarf coconut (Chowghat Green Dwarf) possessing enhanced disease resistance. OMICS: J Integr Biol 24(12):726–742.

Ramesh SV, Arunachalam V, Rajesh MK (2020a) Genomic designing of climate-smart coconut. In: Kole C (ed) Genomic designing of climate-smart fruit crops. Springer, Cham, pp 135–156

Ramesh SV, Hebbar KB, Rajesh MK, Abhin SP, Gangaraj KP, Athul Bobby (2020b) Transcriptome analysis of *Cocos nucifera* L. seedlings having contrasting water use efficiency (WUE) under water-deficit stress: molecular insights and genetic markers for drought tolerance. Proceedings. https://doi.org/10.3390/IECPS2020-08853

Ramesh SV, Krishnan V, Praveen S, Hebbar KB (2021a) Dietary prospects of coconut oil for the prevention and treatment of Alzheimer's disease (AD): a review of recent evidences. Trends Food Sci Technol 112:201–211

Ramesh SV, Rajesh MK, Singh A, Hebbar KB (2021b) Mitochondrial and chloroplast genomes. In: Rajesh MK, Ramesh SV, Perera L, Kole C (eds) The coconut genome. Springer International Publishing, Cham, pp 133–143

Ranasinghe CS (2010) Climate change impacts on coconut production and potential adaptation and mitigation measures: a review of current status. http://www.slcarp.lk/wp-content/uploads/2019/01/Full-Book-Climate-Change_77.pdf

Riangwong K, Wanchana S, Aesomnuk W, Saensuk C, Nubankoh P, Ruanjaichon V, Kraithong T, Toojinda T, Vanavichit A, Arikit S (2020) Mining and validation of novel genotyping-by-sequencing (GBS)-based simple sequence repeats (SSRs) and their application for the estimation of the genetic diversity and population structure of coconuts (*Cocos nucifera* L.) in Thailand. Hortic Res 7:1–16. https://doi.org/10.1038/s41438-020-00374-1

Riedel M, RiedererM BD, Herran A, Kullaya A, Arana-López G, Peña-Rodríguez L, Billotte N, Sniady V, Rohde W, Ritter E (2009) Cuticular wax composition in *Cocos nucifera* L.: physico-chemical analysis of wax components and mapping of their QTLs onto the coconut molecular linkage map. Tree Genet Genome 5:53–69

Rivera R, Edwards KJ, Barker JH, Arnold GM, Ayad G, Hodgkin T, Karp A (1999) Isolation and characterization of polymorphic microsatellites in *Cocos nucifera* L. Genome 42:668–675

Rohde W, Becker D, Kullaya A, Rodriguez J, Herran A, Ritter E (1999) Analysis of coconut germplasm biodiversity by DNA marker technologies and construction of a genetic linkage map. In: Oropeza C, Verdeil JL, Ashburner GR, Cardeña R, Santamaría JM (eds) Current advances in coconut biotechnology. Springer, Dordrecht, pp 99–120

Santhi CKV, Gangaraj KP, Rajesh MK (2018) Genome-wide identification of auxin response factor (ARF) gene family in coconut (*Cocos nucifera* L.). In: Patil S, RoobakKumar A, Krishna Reddy P et al (eds) Book of abstract, 23rd plantation crops symposium, 6–8 March, 2019, Chikkamagaluru, Karnataka, p 51

Santhi CKV, Rajesh MK, Ramesh SV, Muralikrishna KS, Gangaraj KP, Gupta P, Dash PK (2021) Genome-wide exploration of auxin response factors (ARFs) and their expression dynamics in response to abiotic stresses and growth regulators in coconut (*Cocos nucifera* L.). Plant Gene 100344. https://doi.org/10.1016/j.plgene.2021.100344

Singh R, Ong-Abdullah M, Low ETL, Manaf MAA, Rosli R, Nookiah R, Ooi LCL, Ooi SE, Chan KL, Halim MA, Azizi N (2013) Oil palm genome sequence reveals divergence of interfertile species in old and new worlds. Nature 500(7462):335

Subramanain P, Joseph R, Chowdappa P (2018) Impact of the Gaja cyclone on coconut in Tamil Nadu and actionable strategies reviving livelihood. Indian Coconut J 61(8):6–10

Sunoj JVS, Naresh Kumar S, Muralikrishna KS (2013) Variation in total phenols concentration in coconut (*Cocos nucifera* L.) seedlings under elevated CO_2 and temperature in different seasons. In: Sundaresan J, Sreekesh S, Ramanathan AL, Sonnenschen L, Boojh R (eds) Climate change and environment. Scientific Publishers, India, pp 140–149

Voleti SR, Kasturi Bai KV, Rajagopal V (1993) Water potential in the leaves of coconut (*Cocos nucifera* L.) under rainfed and irrigated conditions. In: Nair MK, Khan HH, Gopalasundaran P, Bhaskara Rao EVV (eds) Advances in coconut research and development. Oxford & IBH Publishing, New Delhi, India, pp 243–245

Xiao Y, Xu P, Fan H, Baudouin L, Xia W, Bocs S, Xu J, Li Q, Guo A, Zhou L, Li J (2017) The genome draft of coconut (*Cocos nucifera*). Giga Sci 6(11):gix095

Yang Y, Saand MA, Abdelaal WB, Zhang J, Wu Y, Li J, Fan H, Wang F (2020) iTRAQ-based comparative proteomic analysis of two coconut varieties reveals aromatic coconut cold-sensitive in response to low temperature. J Proteomics 220:103766. https://doi.org/10.1016/j.jprot.2020.103766

Zizumbo D, Been B, Tupinamba EA, Castillo R, Oropeza C (2005) Research on coconut genetic resources in Latin America and the Caribbean. In: Batugal P, RamanathaRao V, Oliver J (eds) Coconut genetic resources. International Plant Genetic Resources Institute, United States, pp 557–569

Zuñiga LC (1953) The probable inheritance of the Makapuno character of coconut. Philipp Agric 36:402–409

Chapter 3
Current Challenges and Genomic Advances Towards the Development Resilient Coffee Genotypes to Abiotic Stresses

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Abstract Climate variability and change are among the major drivers of abiotic stresses and the concomitant vulnerability of agricultural production systems. With the advent of systems biology, the analysis of complex crop-environment interactions through integrated high-throughput approaches, such as genomics, transcriptomics, proteomics, metabolomics, lipidomics, and interactomics, is currently the most assertive strategy to unravel plant development, metabolism, and acclimation

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capabilities, and to implement genomics-assisted breeding programs towards the production of resilient crops. With the sequencing of the coffee reference genome, the last decade has seen a rapid worldwide progress in establishing genomic tools, entering a new era of coffee functional genomics. New genomic tools offer practical toolkits for high-throughput identification of genes and pathways that are key resources for improving the adaptability of coffee crop to the present and future climate change scenarios, using worldwide genetic resources of *Coffea* spp. In this review, we summarize the available coffee genomic resources and discuss their use in the development of new (hybrid) varieties with greater ability to cope with environmental abiotic constraints. To ensure sustainable coffee production, stress-tolerant varieties will be critical in maintaining the coffee bean yield and quality.

Keywords Abiotic stresses · Breeding · Climate changes · Coffee · Crop sustainability · Plant tolerance

3.1 Introduction

Coffee is an important agricultural commodity grown in over 80 countries, with an annual global production of approximately 174 million bags of coffee beans. The entire coffee value chain generates annual revenues over US$ 170 billion in international trade, representing a major income source for several developing tropical countries, and mostly for smallholder farmers (ICO 2020). Brazil, Vietnam, and Colombia account for approximately 63% of the world's coffee production, with Brazil, where both Arabica (*Coffea arabica* L.), and Robusta (*C. canephora* Pierre ex A. Froehner) are cultivated, being the world's largest producer (ICO 2020).

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The genus *Coffea* belongs to the family Rubiaceae and comprises at least 124 species and 17 additional taxa that are naturally distributed across Africa and the Indian Ocean Islands, south and southeast Asia, and Australia (Davis et al. 2011; Guyot et al. 2020). With the exception of *C. arabica,* which is allotetraploid ($2n = 4x = 44$; Rijo 1974) and has a self-fertility rate of approximately 90% (Davis et al. 2011), most coffee species are diploid ($2n = 2x = 22$ chromosomes) and self-incompatible (Nowak et al. 2011; Yu et al. 2011). The origin of *C. arabica* is relatively recent (estimated 10,000 to 665,000 years ago) (Yu et al. 2011; Bawin et al. 2020; Scalabrin et al. 2020) and was derived from a single natural hybridization between ancestor genotypes of *C. eugenioides* S. Moore (the female parent as shown by the analysis of chloroplast genomes) and *C. canephora* (Lashermes et al. 1999). This recent single allopolyploid event, associated with its predominantly autogamous reproduction, resulted in a considerably low genetic diversity in *C. arabica*. Most of the species variability is found in the wild Ethiopian germplasm that represent a reservoir of novel genetic variation for the improvement of *C. arabica* varieties (Scalabrin et al. 2020). *Coffea arabica* is considered a true allotetraploid (Clarindo and Carvalho 2009), i.e., an organism in which bivalents are formed exclusively during meiosis and pairing is restricted to the homologous chromosomes (Stebbins 1947), and rare multivalent associations, characterizing a diploid-like cytogenetic behavior (Soltis et al. 2004).

Owing to limiting factors, such as lack of agronomical inputs, pest outbreaks, climate instability, and abiotic stresses, the genetic potential for growth, development, productivity, and coffee bean quality is not always fully expressed (Bunn et al. 2015; Ovalle-Rivera et al. 2015; Rodrigues et al. 2016; Acidri et al. 2020; Martinez et al. 2020). Abiotic stresses differ with growing season and geographical location, and are the main causes of agricultural yield losses. Abiotic stresses affect the physiological conditions of plants, with considerable impacts at the molecular, biochemical, and morphological levels, causing irreversible damage to the plant (Martinez et al. 2020).

Although *C. canephora* has a lower beverage quality than *C. arabica*, it possesses a higher pest and disease resistance, yield, and caffeine content (Ferrão et al. 2019), thus constituting a potential resource for enhancing stress tolerance in *C. arabica.* Owing to the agricultural and economic importance of *C. canephora*, several countries maintain *C. canephora* germplasm banks (ex situ collections), including Côte d'Ivoire, Cameroon, Uganda, India, Indonesia, and Brazil. In Brazil, public institutions, such as IAC (Agronomic Institute of Campinas), Incaper (Institute of Research, Technical Assistance and Rural Extension of Espírito Santo), Epamig (Agricultural Research Institute of Minas Gerais), and Embrapa (Brazilian Agricultural Research Corporation), Rondônia, serve as *C. canephora* germplasm repositories (Charrier and Berthaud 1985.

Among the most common abiotic stresses are the highly variable environmental conditions, including extreme temperatures, excess light irradiance, drought, inadequate soil nutrient availability, and heavy metal toxicity (Ramalho et al. 2000; Batista-Santos et al. 2011; Martins et al. 2016; Rodrigues et al. 2016; Scotti-Campos et al. 2019; Marie et al. 2020; Scalabrin et al. 2020). These stresses are usually superimposed in nature, thereby increasing their effects and severity (Ramalho et al.

2018b; Dubberstein et al. 2020). Abiotic stresses represent the greatest challenge to the sustainability of coffee plantations, as they affect cellular metabolism and impair the photosynthetic pathway. Additionally, abiotic stresses frequently trigger excessive production of reactive oxygen species, resulting in oxidative stress. Therefore, to prevent undesirable physiological and biochemical changes, it is important that plants possess the capability to overcome these stresses (Ramalho et al. 1998, 2013; Fortunato et al. 2010; Demirel et al. 2020).

Plant response and adaptability to stress is directly related to cellular metabolism as well as other biochemical and physiological processes that activate multiple responses. These consist of complex molecular interactions that are species and genotype dependent (Demirel et al. 2020; Dubberstein et al. 2020) and vary according to the imposed environmental conditions, severity, rate of imposition, and their superimposition. Furthermore, in the context of climate change, recent studies have reported that elevated atmospheric CO_2 promotes the resilience of coffee plants to environmental stress (DaMatta et al. 2019). This was associated with a positive impact on photosynthesis under both low and high irradiance conditions, decreasing both photorespiration rates and oxidative stress, especially under high irradiance (Marçal et al. 2021). Additionally, elevated atmospheric CO_2 mitigates the impact of high temperature on leaf physiology (Martins et al. 2014; Rodrigues et al. 2016) and bean quality (Ramalho et al. 2018a). High atmospheric CO_2 alleviates drought effects at the photosynthetic level (Avila et al. 2020a) while maintaining a greater homogeneous distribution of produced fruits among plant layers (Rakocevic et al. 2021). Therefore, it is important to determine the role of elevated atmospheric CO_2 in managing the effects of temperature and water constraints in coffee production (DaMatta and Ramalho 2006; Ramalho et al. 2014).

The recent progress in plant genomics has facilitated the identification and isolation of important genes and the examination of their roles in regulating yield and stress responses. Although most previous studies have focused on *Arabidopsis thaliana*, recent molecular studies on coffee have shown that the genetic basis of the plant's response to abiotic stresses and elevated atmospheric CO_2 is polygenic, resulting from the expression of multiple genes that regulate physiological and biochemical responses related to photosynthesis, respiration, hormone production, antioxidants, and lipid dynamics (Martins et al. 2017; Acidri et al. 2020; Marques et al. 2020). Therefore, the identification of key abiotic stress-related genes constitutes a significant step towards expanding the knowledge of the genetic architecture of coffee in response to the changing environment (Huang et al. 2020; Marques et al. 2020; Thioune et al. 2020).

Owing to global warming, there has been an increase in temperature and frequency of extreme events, such as severe droughts and floods, which has become a major challenge for the scientific community (Van der Vossen et al. 2015). Regardless of the potential mitigation effect of elevated atmospheric CO_2 (DaMatta et al. 2019), the increase in the number and severity of abiotic stress events will affect coffee crop production in the coming decades, and may impact the genetic resources of coffee species, including *C. arabica*, negatively (Davis et al. 2019; Moat et al. 2019). Therefore, new strategies are being sought to elucidate the physiological, biochemical, and

molecular responses of plants of agronomic interest to environmental disturbances. In the following sections, we will address the current knowledge and tools that can aid in mitigating the impacts of abiotic stresses in coffee plants.

3.2 Genetic Advances in *Coffea*

Molecular data indicate that *C. arabica* was derived from *C. canephora* (CC) and *C. eugenioides* (EE), followed by a polyploidy event, with the genome representation $C_aC_aE_aE_a$ (Yu et al. 2011; Cenci et al. 2012). The 2C value of 2.62 pg for the tetraploid hybrid, *C. arabica* (Noirot et al. 2003; Bennett and Leitch 2011) corroborates its origin. *Coffea arabica* originated from a cross between *C. canephora* Pierre (2C = 1.41 pg) (Clarindo and Carvalho 2009), and *C. eugenioides* Moore (2C = 1.36 pg) (Noirot et al. 2003). The genome description of the three species is given in Table 3.1.

The availability of genome maps and the reduced cost of next generation sequencing (NGS) platforms facilitated the development of highly informative and high-density single nucleotide polymorphism (SNP) and genotyping arrays (Garavito et al. 2016; Merot-L'anthoene et al. 2018; Carneiro et al. 2019), which are powerful research and breeding tools for *Coffea* species. Examples of their application include the development of a highly saturated linkage map for *C. canephora*, with 3,039 markers (Merot-L´anthoene et al. 2018), and the measurement of intra- and inter-specific genetic variability (Carneiro et al. 2019). The new 8.5 k SNP array of coffee (Merot-L´anthoene et al. 2018), comprises 6,824, 7,065 and 6,183 SNPs for *C. arabica*, *C. canephora*, and *C. eugenioides*, respectively. The Coffee Axiom chip—26 K for *C. canephora* (Carneiro et al. 2019) was developed from the resequencing of the representative gene pool of the three species, with a wide SNP distribution

Table 3.1 Comparative genomics of *C. arabica*, and its progenitors (*C. canephora* and *C. eugenioides*)

	Coffea arabica	*Coffea canephora*	*Coffea eugenioides*
Common name	Arabica	Robusta (Conilon in Brazil)	–
Ploidy	Allotetraploid	Diploid	Diploid
Number of chromosomes	$2n = 4x = 44$	$2n = 2x = 22$	$2n = 2x = 22$
DNA content nuclear (value 2C)	2.62 pg[a]	1.41 pg[b]	1.36 pg
Breeding type	Autogamous	Allogamous	Allogamous
Genome size	1.3 Gbp[c]	710 Mb[e]	699 Mb[d]
GC content (%)	36.9585[d]	38.8866[d]	37.153[d]
Gene prediction	46.562[c]	25.574[e]	35.456[d]

[a] Noirot et al. (2003), Bennett and Leitch (2011); [b] Clarindo and Carvalho (2009); [c] Scalabrin et al. (2020); [d] NCBI (2020); [e] Coffee Genome Hub (2020)

across the genome, which was later validated in 296 widely divergent plants of both *C. canephora* and *C. arabica*. The availability of such databases, as well as genomic and moleculares tools, has facilitated considerable advances in molecular genetics, population genetics, and genome-wide selection, towards molecular breeding of coffee. These tools promoted the development of genome-wide selection studies (Alkimim et al. 2020), genome-wide association studies (GWAS) (Sant'Ana et al. 2018; Gimase et al. 2020), marker-assisted selection (Prakash et al. 2011; Caixeta et al. 2015), genetic transformation (Pérez-Pascual et al. 2018; Valencia-Lozano et al. 2019), and gene editing (Breitler et al. 2018). The use of these methods and tools will promote the knowledge of the coffee genome and the direct applications of marker-assisted breeding for stress tolerance.

3.3 Molecular Breeding and Genomics Tools for Improving Tolerance to Abiotic Stresses

Climate change has created challenging conditions for global coffee production, highlighting the importance of cultivars adapted to drought and extreme temperatures. The optimum mean annual temperature range for Arabica coffee is 18–21 °C (DaMatta and Ramalho 2006). Development and ripening of fruits are accelerated at temperatures above 23 °C, leading to quality loss (Camargo 2010). For Robusta coffee, the optimum annual mean temperature ranges from 22 to 30 °C (DaMatta and Ramalho 2006). However, some genotypes of both species can endure temperatures as high as 37–39 °C for a few days without significant negative impacts (Rodrigues et al. 2016; Dubberstein et al. 2020).

The current state of research on abiotic stress in *Coffea* spp. is represented in the word cloud (Fig. 3.1) based on the frequency of the keywords "coffee", "stress", "drought or water stress or heat" in the SCOPUS and Web of Science databases.

Fig. 3.1 Word Cloud ("coffee" and "stress" and "drought or water deficit or heat"). *Source* Bibliometrix (Aria and Cuccurullo 2017)

It is fundamental to expand the knowledge of the genetic control of abiotic stress-related traits (Van der Vossen et al. 2015; Alves et al. 2018). In coffee breeding programs, the use of molecular strategies and genetic tools can accelerate the selection process and maximize genetic gains, thus reducing the development time of new cultivars (Ferrão et al. 2016) and improving the efficiency of identifying stress-tolerant genes and response pathways in *Coffea* species. However, similar to other traits, breeding for abiotic stress-tolerant genotypes depends on the extent of genetic variability and knowledge of the genetic control of the traits. In the topics discussed below, we addressed issues related to the magnitude of variability and molecular and genomic breeding for abiotic stress tolerance. Furthermore, we reviewed advances in molecular studies of the genes regulating drought and heat tolerance in plants, as well as genomic tools and potential research for these traits.

3.3.1 *Variability of* Coffea arabica *and* Coffea canephora *Tolerance to Abiotic Stresses*

Among the two species of economic importance, *C. arabica* has a lower genetic diversity, owing to its predominantly autogamous reproduction system, domestication process, and evolutionary history (Lashermes et al. 1999, 2000; Scalabrin et al. 2020). However, abiotic stress tolerance/sensitivity has been reported in *C. canephora* (DaMatta and Ramalho 2006), and *C. arabica* (Oliveira et al. 2020).

Contrarily, *C. canephora* has a high diversity, and was initially divided into two genetic groups, Congolese and Guinean, originating from Central and West Africa, respectively (Berthaud 1986). Subsequently, the Congolese group was divided into two subgroups, SG1 and SG2, with coffee trees in the SG1 group considered moderately to highly drought-tolerant, while those in the SG2 group were sensitive to drought (Montagnon et al. 1998). The Congolese group was later divided into four subgroups, SG1, SG2, C, and Ug (Cubry et al. 2008). Conilon coffee, which is widely grown in Brazil and comprising both drought-tolerant (D^T) and drought-sensitive (D^S) genotypes, was classified into the SG1 group, (Ferrão et al. 2000; Montagnon et al. 2012). However, a recent genotyping study based on the SNP Coffee 8.5 K array classified the genotypes into eight well-differentiated genetic groups (A, G, B, C, D, E, O, and R) that corresponded to different geographic origins (Merot-L'anthoene et al. 2018). Among the eight groups, six had already been previously identified: group A (North of Congo, South of Cameroon), group B (East of Central African Republic), group C (West of Central African Republic, Cameroon), group D (Guinea, Côte d'Ivoire), group E (Democratic Republic of the Congo, Cameroon) (Dussert et al. 1999; Gomez et al. 2009), and group O (formerly known as Ug group; Uganda, South Sudan) (Cubry et al. 2008; Musoli et al. 2009). The two newly identified groups were group G (North and West of Angola) and group R (South of the Democratic Republic of the Congo).

Regarding *C. arabica*, an extensive study on the genetic diversity of 736 accessions from Ethiopia, Yemen, Africa Oriental, and India, which included cultivated varieties in Asia and Latin American, hybrid genotypes, and landraces, showed that *C. arabica* had a lower variability compared with its ancestors (*C. canephora* and *C. eugenioides*). Tetraploid species, such as *C. arabica*, are subjected to a severe bottleneck effect caused by the low diversity transfer of genetic resources among species of the genus *Coffea* and the form of distribution of specific alleles across the three species (*C. arabica, C. canephora*, and *C. eugenioides*). The vast majority of SNPs identified in *C. arabica* are not shared with either of the parental species, confirming that most of the variation present in *C. arabica* occurred after the polyploidization event and that there have not been any major introgression events between the two parental species and *C. arabica* (Scalabrin et al. 2020).

Knowledge of coffee genetic diversity has become essential in designing breeding strategies and in maintaining ex situ conservation and in situ protection of coffee tree populations. To overcome the challenges of climate change, including global warming, breeding programs aim to explore the genetic diversity of *C. arabica* populations beyond the Ethiopian collections. There is an urgent need to protect and conserve the *C. arabica* populations and other wild relative species that could become extinct due to climate change (Davis et al. 2019; Moat et al. 2019). Another way of increasing the genetic diversity of *C. arabica* is through introgression between the tetraploid species and its diploid parents or wild relatives that have higher levels of genetic diversity. This strategy was applied for enhancing tolerance to coffee leaf rust caused by the fungus *Hemileia vastatrix* in the Timor Hybrid (Clarindo et al. 2013; Herrera et al. 2014), as well as in other Arabica-Liberica hybrids.

Additionally, drought tolerance in coffee was also studied based on the sequence variability of the *DREB1D* gene (Alves et al. 2018). The genetic diversity of the *loci* demonstrated distinct haplotypes for the promoter and coding regions of *DREB1D* in 38 drought-tolerant, and sensitive genotypes (11 *C. canephora*, 26 *C. arabica,* and one *C. eugenioides*). For *C. canephora,* the nucleotide diversity was higher in the promoter than in the coding regions, and cluster analysis grouped the genotypes in agreement with the population structure, classifying the genotypes into five genetic subgroups (SG1, SG2, B, C, and UW). The three most relevant divergence regions were detected in *CcDREB1D* and *CeDREB1D*. For *C. arabica,* few haplotypes were detected and there were only two in common with that of *C. canephora.* The main diversity of *CaDREB1D* was observed in the sub-genome *CaCe*. A low diversity was identified in the coding region of the *DREB1D* gene, where the non-synonymous polymorphisms were neutral and the synonymous polymorphisms corresponded to 56% of the variations in the sequences (Alves et al. 2018).

In addition to conventional strategies, coffee breeding programs aim to identify environmental stress-tolerant genes that can be used in developing new cultivars. In the last decade, several studies have identified a complex network of gene responses involved in the perception and recognition of stress signals by the activation of stress-induced genes in *Coffea* spp. These stress-induced genes are involved in different plant processes to protect cells against water shortage, including the synthesis of important proteins, adjustments of the osmotic potential of plants to ensure the

maintenance of cell turgor pressure, and the regulation of target genes and transcription factors (dos Santos et al. 2011; de Carvalho et al. 2014; Nobres et al. 2016). Additionally, a considerable number of genes linked to coffee physiological and biochemical stress responses were recently found to be affected by elevated atmospheric CO_2, with a significant upregulation of photosynthetic, antioxidant, and lipid related genes (Marques et al. 2020). Under elevated atmospheric CO_2, *C. canephora* 'Conilon (CL153)' expresses genes associated with general biological processes, and to a lower extent, with abiotic stress responses. In contrast, *C. arabica* 'Icatu showed a greater upregulation of genes linked to plant tolerance and adaptation to abiotic factors, such as genes involved in oxidative stress response (Marques et al. 2020). A group of genes closely involved in the regulation of the membrane lipid matrix, chloroplast/thylakoid organization, and photosystem (PS) II repair were also upregulated in Icatu. This supports a greater photosynthetic performance under heat (Rodrigues et al. 2016) and drought (Avila et al. 2020a) stress conditions in plants grown under elevated CO_2 than under ambient CO_2, with remarkable differences found between genotypes (Marques et al. 2020).

Therefore, it is important to examine the performance of various coffee genotypes under different environmental conditions to strengthen in situ management. It is also necessary to examine the expression patterns of several 'novel' genes coding many of the uncharacterized/putative proteins of coffee (Marques et al. 2020). The functional annotation of these uncharacterized genes is still a substantial challenge but would be critically important in deciphering coffee responses to future environmental conditions.

3.3.2 Advances in QTLs Detection and Genome-Wide Selection for Stress Tolerance in Coffee

Through genome-wide association studies (GWAS), the genome regions with the greatest effect on a given trait can be identified. There are no reports of GWAS studies for drought and heat tolerance in *C. arabica* and *C. canephora.* However, the first GWAS in *C. arabica,* using a population of 107 accessions from Ethiopia, identified 21 markers associated with bean lipid composition as well as diterpenes content, which are compounds that affect the beverage quality of coffee (Sant'Ana et al. 2018). GWAS was also used to identify resistance genes against *Colletotrichum kahawae* infection (Gimase et al. 2020).

Recently, the efficiency of GWAS was evaluated in a population of 165 clones of the Conilon and Robusta varietal groups, and intervarietal hybrids originating from crosses between Conilon and Robusta. For some major phenotypic traits, a moderate to high accuracy was estimated, and the selection cycle was shortened from six to three years, with a selective efficiency ranging from 22 to 146% (Alkimim et al. 2020). Although this study was not directly related to abiotic stresses, GWAS has been proven to be a useful and promising tool for the breeding of *C. canephora*, as

it can be used to identify reliable molecular markers for breeders, shorten selection cycle, and improve selection efficiency per unit time. Overall, GWAS could be useful for future studies on drought and heat tolerance in coffee plants.

3.4 Advances in Genomics Studies of *Coffea arabica* Under Abiotic Stress Conditions

Coffee breeding programs are mainly focused on identifying environmental stress tolerance genes and transcription factors to enable the development of new cultivars (dos Santos et al. 2011; de Carvalho et al. 2014; Nobres et al. 2016). Raffinose family oligosaccharides (RFOs), which include raffinose, stachyose, and verbascose, can be synthesized and accumulated in high quantities when a coffee plant is subjected to abiotic stress. RFOs are known to serve as desiccation protectant against cold, salinity, osmotic, and drought stress. RFOs are compatible osmotic compounds that are involved in plant stress tolerance response. Additionally, RFOs possess antioxidant properties, stabilize cell membranes (e.g., of thylakoids), and are involved in plant carbon partitioning strategies (Dos Santos and Vieira 2020). This seems to be the case in *C. arabica* 'Catucaí IPR 102' that showed considerable increases in raffinose and stachyose content under cold conditions. However, *C. canephora* 'Conilon (clones 02 and 153)' showed an increase in raffinose content under cold conditions only (Partelli et al. 2010).

Galactinol synthase (*GolS*) is one of the major genes involved in the biosynthesis of RFOs, and three isoforms have been identified and characterized (*CaGolS1*, *2* and *3*) in coffee trees under abiotic stress conditions (Dos Santos et al. 2011). Additionally, it was suggested that *CaM6PR*, *CaPMI,* and *CaMTD* transcripts, which are involved in mannitol biosynthesis, are modulated in distinct ways in response to abiotic stress (De Carvalho et al. 2014). For example, during heat stress conditions, *CaM6PR*, *CaPMI*, and *CaMTD* are expressed differently. This study further suggested that during drought and salt stress, leaves triggered an increase in the expression of the major genes involved in the mannitol biosynthesis. Both studies indicate that RFOs regulating genes participate in cellular osmoprotection in coffee under adverse environmental conditions. These genes possess great potential for introgression in breeding programs.

A drought tolerance transcriptome study, using high-throughput sequencing (HTS), was performed on two different *C. arabica* cultivars (Rubi MG1192—drought-susceptible; IAPAR59—drought-tolerant), and the results indicated that differences in phenotypic behavior between cultivars were associated with the transcriptome profiles under drought conditions (Mofatto et al. 2016). These authors suggest that the expression of *CaSTK1*, *CaSAMT1*, *CaSLP1*, and *CaMAS1*, were differentially regulated under drought conditions, due to the contribution of the homologous genes expressed in the *C. arabica* genome, which is composed by two

subgenomes (*CaCe* and *CaCc*). The divergence between the subgenomes may indicate that there is a mechanism preventing the homogenization of the subgenomes (Vidal et al. 2010). In this sense, with based on the above information, further studies should be performed to examine the contribution of *CaCc* and *CaCe* sub-genomes to the complete *C. arabica* genome.

Among the abiotic stresses, salt stress is one of the least explored in coffee. The transcriptome of *C. arabica* seedlings grown under salt stress conditions identify several upregulated genes, including *Cc00_g13890, Cc10_g04710, Cc02_g14240, Cc04_g05080, Cc08_g11060,* and *Cc06_g01240* (Haile and Kang 2018). Among these genes, *Cc08_g11060* and *Cc00_g13890* are putative WRKY transcription factors, which are reported to be involved in plant response to various stresses (Zheng et al. 2019).

Among the nutrients most used in crop production, nitrogen (N) is the most important element used for the fertilization of the coffee tree. High-throughput sequencing (HTS) technologies were used to analyze the mRNAs and microRNAs (miRNAs) profiles of the roots of *C. arabica* in response to N fertilization (Dos Santos et al. 2019). Three ammonium transporters (AMTs—*CaAMTa*, *CaAMTb*, *CaAMTc*) and three nitrate transporters (NRTs—*CaNRTa*, *CaNRTb*, *CaNRTc*) were identified by transcriptional analysis, and miRNAs involved in N stress response also validated using RT-qPCR. Among the identified miRNAs, miR169 and miR171, which are part of an evolutionarily conserved miRNA family in plants, were involved in abiotic stress responses (Noman et al. 2017; Rao et al. 2020). However, most of the identified putative microRNA sequences were mainly for *C. canephora* and *C. arabica* (Mhuantong and Wichadakul 2009; Szcześniak et al. 2012; Loss-Morais et al. 2014; Chaves et al. 2015; Bibi et al. 2017). A catalog of 6,976 noncoding RNAs (ncRNAs) in leaves, including 92 miRNAs, which were identified using RNA sequencing, was published for *C. canephora* (Lemos et al. 2020). In this context, the discovery of these new miRNAs opens the possibility of a molecular characterization of pathways and components that were regulated during the plant's response to abiotic stress.

Martinez et al. (2020) reported that the genes involved in N absorption (*NRT1.2* and *NRT3.2*) and metabolism (*NIA2*—Nitrate Reductase, *GLN1.3*—Glutamine Synthase and *GLT1*—Glutamine Oxoglutarate Aminotransferase) were differentially expressed under water stress and N deficiency in two *C. arabica* cultivars (Catuaí Amarelo IAC62 and Mundo Novo IAC379-19). The result of the study indicated that water availability modified the expression of *NRT1.2*, *NRT3.2*, *NIA2*, and *GLT*, which showed high levels of expression in the roots. Evidently, water stress limited the coffee plants ability to assimilate nitrogen by inhibiting the activities of enzymes involved in nitrogen metabolism, which is in line with earlier reports that the activity of leaf nitrate reductase is highest (and greater leaf nitrate concentration) during warm and wet season (Carelli et al. 2006). To improve the N assimilation capacity of plants under high temperatures and water shortage, the understanding of the effects of abiotic stresses on nitrogen metabolism is of fundamental importance.

Regarding thermal tolerance, intraspecific differences in transcriptional pathways and physiological responses under high temperature conditions were reported in the commercial genotypes of Catuaí (IAC144 and Acauã). Intraspecific differences were observed at elevated temperature and 52 differentially expressed genes were

identified. The identified genes can be used as biomarkers for the development of high temperature tolerant genotypes (Oliveira et al. 2020). Among the differentially expressed genes, some were involved in the regulation of protective molecules associated with oxidative stress management (e.g., antioxidative enzymes), and protection (e.g., chaperonins and heat shock proteins), both in *C. arabica* and *C. canephora* genotypes under heat, cold and drought conditions (Martins et al. 2016; Ramalho et al. 2018a, b).

It is important to mention that the introgression of new candidate genes in breeding programs will contribute in increasing the genetic diversity among cultivars for the breeding of stress-tolerant cultivars. In this context, several genomes resources for *C. arabica* have recently been published. A research team from California University published a fragmented sequence of the *C. arabica* genome on the public database, Phytozome.net (accession Geisha; van Deynze et al. 2017). Additionally, in 2018 the National Center for Biotechnology Information (NCBI), made available the complete genome sequence of *C. arabica* cv. Caturra (https://www.ncbi.nlm.nih.gov/genome/gdv/?org=coffea-arabica&group=lamiids) and *C. eugenioides* (https://www.ncbi.nlm.nih.gov/assembly/GCF_003713205.1). The genome of *C. arabica* 'Bourbon' is also available (Scalabrin et al. 2020). Additionally, the complete genome sequences of *C. canephora*, *C. eugenioides*, and *C. arabica* from the Arabica Coffee Genome Consortium (ACGC, Mueller et al. 2015) will soon be available. These genomes can boost the identification and characterization of molecular components triggered by abiotic stresses, which will improve the understanding of the tolerance mechanisms at the transcriptional and physiological level. Additionally, the available genome will be useful in identifying genes with biotechnological potential for application in genetic transformation strategies towards improving plant responses to abiotic stresses (Fig. 3.2), and in developing stress-tolerant cultivars.

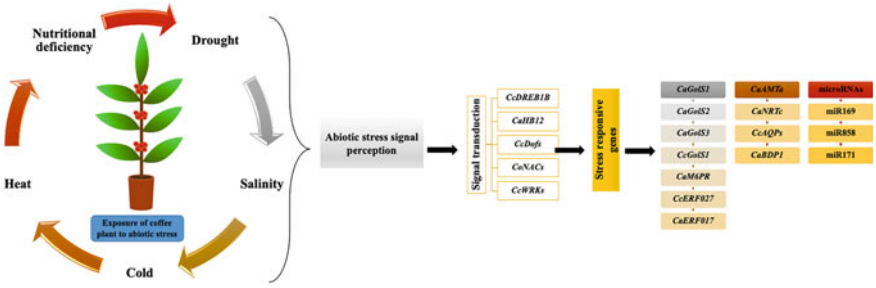

Fig. 3.2 A simplified scheme of mechanism for abiotic stress tolerance in coffee plants. Briefly, abiotic stress triggers a cascade of events and signals, initiated from the perception of stress, and culminated in the activation of genes involved in the stress response process

3.5 Genomics Approaches to Understand Abiotic Stress Responses in *Coffea canephora*

Among coffee species, *C. canephora* is most valuable in the world coffee market, because of its chemical composition, bean quality (Dankowska et al. 2017), and high tolerance to coffee leaf rust (CLR) caused by *H. vastatrix* Berk. and Broome (Zambolim 2016). Additionally, *C. canephora* has a deeper and well-developed root system than *C. arabica*, making it more drought-tolerant (DaMatta and Ramalho 2006). These characteristics are of considerable importance, as adverse environmental conditions, such as prolonged drought, can impair coffee growth and development, and negatively affecting flowering, fruiting, bean formation, yield, and quality of coffee beans (DaMatta et al. 2010; Vinecky et al. 2017; Imbach et al. 2017).

Coffea canephora was the first species of coffee with its genome sequenced through the collaboration between Genoscope, the Institute de la Recherche pour le Développement (IRD) and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), financed by the French Agency for Research (ANR). The sequenced genotype (diploid, $2n = 2x = 22$ chromosomes = 710 Mb) was a doubled haploid plant (DH200-94 access) produced by IRD from the clone, IF200, based on haploid plants occurring spontaneously in association with polyembryony. The complete genome of *C. canephora* consisted of 25,574 protein-coding genes and a high proportion (~50%) of repeated sequences (transposable elements) (Denoeud et al. 2014). The complete genome of *C. canephora* can be accessed at "Coffee Genome Hub" (Dereeper et al. 2015). The genome has been used to characterize important genes involved in abiotic and biotic stress tolerance.

For example, 24 genes from the *Dof* family were identified in the *C. canephora* genome (Garcia et al. 2018). Dof domains are transcription factors that perform a variety of biological processes in diverse organisms ranging from single-celled, such as *Chlamydomonas*, to higher plants, including monocots and dicots (reviewed by Yang et al. 2018). The expression patterns of the different members of the *Dof* gene family in coffee showed that majority of the genes are specifically expressed in root and leaf tissues. These results provide insights into the molecular mechanisms of *CcDofs* genes in the abiotic stress response of *C. canephora.*

The regulatory role of members of the *DREB* subfamily under stress conditions have also been the target of studies in *C. canephora.* Among these members, the expression of *CcDREB1D* was higher in the leaves of stress-tolerant clones (14) of *C. canephora* compared with that in leaves of the sensitive clone (22), under water stress conditions (Marraccini et al. 2012). Therefore, it can be inferred that *CcDREB1D* participates in drought response in coffee plants (Alves et al. 2017).

Evidence indicates that the *GolS* gene may play an important role in stress tolerance and in the translocation of photosynthesis products (Sengupta et al. 2015). Dos Santos et al. (2015), evaluated the transcriptional profile of *CcGolS1* gene in two *C. canephora* clones (clone 14) and (clone 109A), and reported that the expression of *CcGolS1* was differentially regulated by water stress in the two clones.

Another study evaluated the expression of *CcHSP90-7*, *CcDH1a*, *CcDREB1*, *CcMYB102*, *CcNCED3*, and *CcATAF1* in response to a sudden drop in relative humidity at three different temperatures (27, 35, and 42 °C) using *C. canephora*

leaves (Thioune et al. 2017). The expression of *CcHSP90-7* gene increased with an increase in temperature up to 42 °C and a rapid decrease in relative humidity. However, the expression of *CcDH1a* was higher at 27 °C compared with the expression pattern at the two other temperatures, whereas the expression of *CcDREB1* was higher at 42 °C. The expression of *CcMYB102* was not affected by temperature change. Studies have shown that *CcNCED3* is involved in rate limitation during abscisic acid synthesis (González and González-Vilar 2001; Tan et al. 2003). The expression of *CcNCED3* increased with an increase in temperature from 27 to 35 °C. Additionally, the expression of these genes was closely related to the rapid decrease in relative humidity.

Aquino et al. (2018) also showed that the three promoter haplotypes (HP15, HP16 and HP17) of *CcDREB1D* exhibited divergences in several single nucleotide polymorphisms and insertions/deletions in the drought-tolerant (HP15/HP16) and drought-sensitive (HP15/HP17) clones of *C. canephora*. This result clearly supports the idea that the molecular mechanisms underlying the expression of DREB gene during abiotic stress are probably highly conserved in tobacco and coffee plants (Aquino et al. 2018). In another study the *CcDREB1D* gene is also was considered important in abiotic stress responses in *C. canephora* and *C. arabica* (Torres et al. 2019).

Recently, 31 *DREB*-like orthologous genes were identified in the *C. canephora* genome, with *CcDREB1B*, *CcRAP2.4*, *CcERF027*, *CcDREB1D,* and *CcTINY* being differentially expressed in the leaves of drought-tolerant *C. canephora* (Torres et al. 2019). Particularly the *CcERF016*, *CcRAP2.4*, and *CcDREB2F* genes were expressed in the roots of the drought-sensitive *C. canephora* clone (22).

Furthermore, using the *C. canephora* genome database, Dong et al. (2019) identified and examined the expression patterns of members of the NAC gene family (*CocNACs*). Among 63 identified NAC genes, four *CocNACs* were differentially expressed during the different developmental stages of coffee beans. Additionally, 49 WRKY genes (*CcWRKYs*) were identified and their responses to cold stress were examined (Dong et al. 2019). During the cold acclimation period, 14 *CcWRKYs* were induced. Under 4 °C treatment, 17 *CcWRKYs* were upregulated and 12 *CcWRKYs* were downregulated.

As earlier mentioned, adverse conditions such as drought, salinity, low temperature, and other abiotic stresses can limit plant growth, development, and productivity considerably. Several studies demonstrated the involvement of aquaporins (AQPs) in abiotic stress response (reviewed by Sade and Moshelion 2017; Zhou et al. 2019). Yaguinuma et al. (2021) analyzed the genome database of *C. canephora* and identified 33 putative *AQPs* genes.

Additionally, the effect of water stress on the expression of genes belonging to the *CcPIPs* and *CcTIPs* subfamilies in the leaves of two genotypes of *C. canephora* (clone 14: drought-tolerant and clone 109A: drought sensitive) have been examined. The result showed that the expression levels of three *CcPIPs* (*CcPIP 1,2*, *CcPIP 2,3*, and *CcPIP 2,4*) and two *CcTIPs* (*CcTIP 1,2* and *CcTIP 2,1*) isoforms were affected in both genotypes under water stress conditions, suggesting their potential roles in water stress response in coffee plants. Avila et al. (2020b) reported that hydraulic

conductance in *C. arabica* plants was unaffected by drought under high atmospheric CO_2, which was associated with the high expression of several aquaporin genes, indicating that high atmospheric CO_2 regulates drought response in coffee plants.

It is necessary to emphasize that drought responses in plants depend on various factors, including genotypes. The capacity of plants to adapt to an ever-changing environment determines their capability to respond to future environmental conditions. Diurnal and seasonal cycles in climate conditions force plants to adjust their metabolism, and stress memory allows them to select the most appropriate response to certain changes in the environment (Fleta-Soriano and Munné-Bosch 2016). Guedes et al. (2018) identified 49 "memory genes" in the drought-tolerant clone (120) of *C. canephora* under drought stress. The memory genes were mainly linked to the ABA pathway, protein folding, and biotic stress response. Taken together, the results of these authors revealed that transcriptional memory can alter the expression of drought-responsive genes and contributes to drought tolerance in these species.

Recently, Thioune et al. (2020) examined the transcriptional profile of a clonal variety *C. canephora* (FRT07) under water stress conditions. The result showed that 13,642 genes were differentially expressed in the leaves of the species under water stress conditions and during recovery at 27 °C, with the greater variation in gene expression observed under the most severe drought conditions.

Advancements in sequencing technologies and assembly of chloroplast genomes could be useful in studies on coffee plants (Daniell et al. 2016; Guyeux et al. 2019). The complete chloroplast genome sequence of *C. canephora* was recently published (Wu et al. 2017). The genome contained 131 genes, among which were 18 double copies genes, including protein-coding genes (*ndhB*, *rpl2*, *rp*l23, *rps12*, *rps7*, and *ycf2*). The complete chloroplast genomes of 52 *Coffea* species, including two *C. arabica*, two *C. eugenioides*, and six *C. canephora* accessions, revealed the high diversity of *C. canephora* and a distinct evolutionary origin between the chloroplasts of *C. canephora* and *C. arabica* (Charr et al. 2020). Additionally, Park et al. (2019) published the complete chloroplast genome of *C. arabica* (named as CH_3) under cold treatment, to identify cold resistance genes in coffee. This study revealed the presence of three single nucleotide polymorphisms and three insertion and deletion, and based alignment of chloroplast (NC_008535). The findings of the above study present genetic resources for biotechnological applications, which can help improve the understanding of cold stress response and tolerance in coffee plants.

3.6 Research Involving *Coffea eugenioides*

Despite the importance of understanding the expression of genes from the subgenomes of *C. arabica*, and the relationship with the diploid ancestors, few studies have examined *C. eugenioides*. To provide further information on *C. eugenioides*, NGS methods have been used to study the transcriptome profile of *C. eugenioides*.

The transcriptomes of the leaves and fruits of *C. eugenioides* were first examined by Yuyama et al. (2016). The data revealed possible genes that could contribute

to certain characteristics of *C. arabica* species. Additionally, genes of *C. eugenioides* have been validated and may be future candidates in genetic studies of *Coffea* spp.; this will aid in elucidating the expression of genes from *C. arabica* subgenomes. In 2018, the *C. eugenioides* genome, which contained 44,358 transcripts, became publicly available in the NCBI database (https://www.ncbi.nlm.nih.gov/genome/gdv/?org=coffea-eugenioides&group=lamiids). Understanding the molecular responses in *C. eugenioides* is also important because through "omics" technologies, new genes can be identified with desirable agronomic characteristics.

3.7 Selection of Reference Genes for Studying Abiotic Stress Conditions in *Coffea*

Several research groups have addressed the importance of selection and proper validation of reference genes under environmental adverse conditions in several crop species (Tang et al. 2017; Chen et al. 2019; Zeng et al. 2020), including coffee (Cruz et al. 2009; Goulao et al. 2012; de Carvalho et al. 2013; Martins et al. 2017). These reference genes, also known as housekeeping genes, have constitutive expression in different tissues and cells under different conditions (Guénin et al. 2009). An ideal reference gene must have a stable expression in all tissues and should not be affected by experimental treatments (Thellin et al. 1999). Traditional reference genes used in plant research includes glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), 18S ribosomal RNA (*U18S*), ubiquitin (*UBQ*), SAND family protein gene (*SAND*), tubulin beta gene (*TUB*), elongation factor 1α (*EF1α*).

Eight reference genes (*GAPDH*, *rpl39*, *UBQ10 UBI9*, *psaB*, *AP47*, *PP2A*, and *S24*) were identified in the first study on reference genes in coffee under abiotic conditions (Cruz et al. 2009). Their expression was validated in control *vs*. drought-stressed leaves, control *vs.* drought-stressed roots, in the leaves of three different cultivars, and in four different coffee organs. The results indicated that *GAPDH*, *UBQ10*, and *S24* were the most appropriate reference genes, exhibiting high stability in the leaves of the control and drought-stressed coffee plants (Cruz et al. 2009).

A set of ten potential reference genes (*RPL39*, *UBQ10*, *S24*, *GAPDH*, β-tubulin-*β-TUB*, tonoplast intrinsic protein-*TIP41*, elongation factor 1-*EF1*, elongation factor 1-alpha-*EF1α*, RNA polymerase-II transcription factor-*RPII*, and malate dehydrogenase-*MDH*), were also validated in coffee plants during abiotic stress treatments (nitrogen starvation, salinity, and heat stress) (de Carvalho et al. 2013). Two genes (*MDH* and *EF1*) were recommended for use as reference genes; however, the two genes are not normally used as internal control for normalization in RT-qPCR involving other plant species.

Goulao et al. (2012) found that *UBQ10*, *GAPDH*, *ACT*, and *EF1A* were suitable as reference genes under cold stress, while *GAPDH*, *ACT*, *EF1A*, and *Apt* were suitable

under drought stress. Additionally, *UBQ10*, *GAPDH*, *ACT*, and *elf-4A* were suitable as reference genes under multiple stress conditions. Thus, providing additional reference genes for genomic studies in *Coffea* spp.

Studies have also been performed to identify suitable reference genes under future environmental conditions of elevated atmospheric CO_2 and warming (Martins et al. 2017). A total of 10 genes (actin-*ACT*, *GAPDH*, eukaryotic initiation factor 4α-*Elf4A*, elongation factor 1α-*EF1A*, cyclophilin-*CYCL*, *MDH*, 40S ribosomal protein subunit 15A-*S15*, DNAJ-like-*DNAJ*, ubiquitin-conjugating enzyme E2-*UBQ2*, an alpha tubulin- *a-TUB*) were identified as suitable reference genes. *UBQ2* was most indicated for temperature, while *Elf4A* and *ACT* were most stable under multiple stress conditions (Martins et al. 2017).

It is worth mentioning that the expression patterns of genes encoding for key enzymes involved in reactive oxygen species (ROS) scavenging were recently studied, including APX for H_2O_2 scavenging [(APXc (cytosolic), APXm (membrane-bound), and APXt + s (stromatic)], for energy dissipation in the photosystems through the synthesis of ZEA by violaxanthin de-epoxidase, VDE (VDE2), and one class III peroxidase (PX4) (Ramalho et al. 2018b). These findings highlight the key role of the antioxidative system in response to drought, and cold stress in *Coffea* spp. Drought mostly promoted enzyme activity, whereas cold enhanced the complementary synthesis of both enzyme and non-enzyme antioxidants, with the latter probably relying more on non-enzyme molecules under cold, when enzyme reactions would be repressed.

Considering the importance of the potential impact of abiotic stresses on coffee plants, these studies are crucial with regard to the small contribution of the optimal reference genes that should be used in treatment-specific studies.

3.8 Epigenetics Insights into Abiotic Stresses in the *Coffea* Genus

Epigenetic changes are influenced by abiotic and/or biotic factors, resulting in plant acclimation to different environmental conditions. These modifications provide a basis for a stress memory, which apart from preparing the progenies against future stress events, allows plants to respond efficiently to a recurrent stressful condition. A known mechanism that modulates gene expression during abiotic stresses is the transcriptional memory, which can be defined as any structural, genetic, or biochemical modification resulting from a stress condition that allows the plant to "remember" the stress it was affected with in subsequent conditions (Kinoshita and Seki 2014). In the field, the occurrence of repeated drought spells is common, which can modify plant response and induce acclimation (Galle et al. 2011; Fleta-Soriano and Munné-Bosch 2016).

Recent research has shown that coffee plants exposed repeatedly to drought events adapt better to water restriction than those exposed to drought stress a single time

(Menezes-Silva et al. 2017). Two *C. canephora* clones, one drought-tolerant (120) and drought-sensitive (109), were subjected to one and three periods of water stress. Transcriptome analysis of leaves detected 826 responsive genes in the tolerant and 135 genes in the sensitive clone, which contained a higher amount of secondary antioxidant compounds (Guedes et al. 2018). In the same study, it was discovered that miRNAs and MYB modulated drought responses and that the transcriptional memory of water deficit in the drought-tolerant clone was linked to abscisic acid (ABA)-related genes. The transcriptional memory observed in clone 120, enabled the plant to acclimatize to adverse conditions by triggering stress-related genes. However, when clone 109, with only one "memory" event, was exposed to the third stress event, the programmed cell death mechanism was activated (Guedes et al. 2018).

Epigenetic diversity can thus be exploited as a new source of phenotypic variation to improve the plants ability to cope with the changing environments, thus ensuring high crop yield and quality (Gallusci et al. 2017). In other crops, similar approaches and tools have been developed to assess and quantify the effects of epigenetic variations on the responses to environmental constraints (Baulcombe and Dean 2014), as well as for respiration, yield components, and quality (Hauben et al. 2009; Long et al. 2011; Chen and Zhou 2013), offering practical perspectives for breeders. In *Coffea*, epigenetic studies on drought and heat tolerance are still in the developmental stages.

3.9 Current and Future Challenges of Genetic Engineered Coffee Plants

Abiotic stresses cause morphological, physiological, biochemical, and molecular changes in plants, and consequently lead to yield losses (Osakabe et al. 2011). Owing to the low genetic diversity of *C. arabica*, traditional breeding for abiotic stress tolerance is difficult (Scalabrin et al. 2020). Genes for abiotic stress tolerance, including heat, cold, drought, and salinity tolerance, are frequently polygenically inherited quantitative traits that require a prolonged period for successful transmission through conventional breeding (van der Vossen et al. 2015). To this end, the use of genetic engineering tools will be necessary for the rapid development of abiotic stress-tolerant coffee varieties.

In the last 10 years, efforts have been made to improve genetic transformation in coffee plants, and several research groups were successful in producing transgenic plants. Pro-embryogenic cell induced from leaves were the preferential explant for *Agrobacterium*-mediated transformation in *C. arabica* (Ribas et al. 2011; Alves et al. 2017) and *C. canephora* (Breitler et al. 2018; Pérez-Pascual et al. 2018), as well as for the biolistic delivery system in *C. arabica* (Albuquerque et al. 2009; Barbosa et al. 2010; Valencia-Lozano et al. 2019). In all the cases, transgenic coffee plants were regenerated by somatic embryogenesis. Most of the protocols developed thus far have focused on optimizing the process of coffee transformation or in a few cases,

allowed for functional gene validation. Although several publications have reported that coffee transformation is feasible, owing to the lack of public and private interests, public concerns regarding transgenics, and the high costs involved in the regulatory approval for commercialization of transgenic plants, coffee transgenic plants are yet to be commercialized.

To identify candidate genes for the development of transgenic coffee plants, contrasting genotypes related to drought tolerance have been evaluated under water limitation. *CcDREB1B*, *CcRAP2.4*, *CcERF027*, *CcDREB1D*, and *CcTINY* were upregulated in leaves of drought-tolerant *C. canephora* plants, while *CcERF016*, *CcRAP2.4*, and *CcDREB2F* were upregulated in the roots of drought-sensitive varieties (Torres et al. 2019). Aquaporin genes, including *CcPIP2;3*, *CcTIP1;2*, and *CcTIP2;1*, were highly expressed in the leaves of drought-tolerant *C. canephora* clones subjected to water stress (Yaguinuma et al. 2021). Therefore, such genes are suitable candidates for the engineering of drought-tolerant coffee plants.

The promoter regions from the *DREB1D* transcription factor were functionally validated in transgenic coffee (Aquino et al. 2018). The promoters were cloned from drought-tolerant and susceptible *C. canephora* varieties and three haplotypes, including HP15, HP16, and HP17, were merged with the *uidA* gene and used for generating transgenic *C. arabica* plants using *Agrobacterium tumefaciens*-mediated gene transfer. Among the haplotypes, the expression of HP16 increased under water stress (Alves et al. 2017). GUS expression was also higher in pHP16-transgenic plants under low humidity, high irradiance, and ABA stress than in the other two haplotypes (Torres et al. 2019). Abiotic stresses also upregulated the expression of endogenous genes, such as *CaERF017* (low temperature and relative humidity), *CaERF053* (low humidity), and *CaERF014* (high temperatures) (Torres et al. 2019). However, when these constructs were used to transform tobacco, the expression of HP17 instead of HP16 increased when the transgenic plants were subjected to water stress and heat shock treatments (Aquino et al. 2018). These results confirm that it is necessary to test these genes and promoters for functional validation in homologous rather than in heterologous systems. The functional validation of promoters by transgenesis is important to identify suitable promoters for the development of abiotic stress-tolerant transgenic coffee varieties.

Novel breeding technologies, such as genome editing, are promising in the development of genetically engineered coffee plants. Genetically edited plants are considered non-GE plants in several countries, including Brazil (since 2018 in Brazil). This could facilitate the acceptance of new biotechnological varieties and reduce the cost of production.

Different site-directed nucleases for genome editing have already been tested. However, greater success was achieved using CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein) owing to its simplicity, efficiency, reduced cost, and flexibility, making this technology a powerful tool for the generation of genetically engineered plants (Vats et al. 2019). CRISPR/Cas9 technology is based on the type II prokaryotic adaptive immune system from bacteria or archaea that prevent invasion by phages. The most used CRISPR/Cas9 system is derived from *Streptococcus pyogenes*, which includes a

precursor CRISPR RNA (pre-crRNA), trans-activating crRNA (tracrRNA), and Cas9 nuclease (reviewed by Vats et al. 2019).

Genome editing in coffee using CRISPR/Cas9, was first achieved in the diploid ($2n = 2x = 22$) self-sterile *C. canephora* (Breitler et al. 2018). In this study, the disruption of the phytoene desaturase gene (*CcPDS*) was targeted and the edited lines were obtained by co-cultivating pro-embryogenic cells with *Agrobacterium tumefaciens.* The PDS gene has been frequently used in several species, including trees, as a proof of concept in the CRISPR editing system due to an easy recognition phenotype whose mutation disrupts chlorophyll biosynthesis, allowing for visual identification (Bewg et al. 2018). The disruption of PDS gene in coffee resulted in plants with small lanceolate internodes in leaves with abnormal organization and pigmentation, and rosette-like phenotype. The efficiency of gene editing was approximately 30.4%, with 22.8% heterozygous and 7.6% homozygous mutants (Breitler et al. 2018).

Genome editing in polyploid plants, such as *C. arabica* could be difficult since more than two copies are present on any targeted locus. Hence, for polyploid plants, several lines will have to be screened and phenotyped after the GE experiment to identify the edited lines (Aglawe et al. 2018). CRISPR technology can also be used to induce mutations in allelic series in multigenic families to investigate functional redundancy and the allele dosage (Bewg et al. 2018). For woody perennials plants, such as coffee, long-term stability edited lines can be generated through the vegetative propagation of T0 transformants (Bewg et al. 2018). The clonal propagation by somatic embryogenesis is a well-established technique for coffee (Campos et al. 2017; Etienne et al. 2018). Cloning T0 edited lines is necessary to maintain somatic mutations and to accelerate the process of obtaining new varieties in coffee.

In summary, in recent years, much progress has been made in tissue culture procedures, transformation methods, and the availability of genomic tools for *C. arabica* and *C. canephora*, thus providing numerous opportunities for the development of abiotic stress-tolerant coffee varieties.

3.10 Final Remarks

The studies reviewed herein have shown that molecular genetic analyses have contributed in the identification of traits and genes related to abiotic stress tolerance and resistance in coffee. However, most of the studies were focused on the two mainly cultivated species *C. arabica* and *C. canephora.* In recent years, advances in sequencing technology have facilitated complete sequencing of the genomes of *C. arabica*, *C. eugenioides*, and *C. canephora*, and high-throughput genotyping of hundreds of species. Moreover, such large data sets could be used in breeding programs for the introgression of genes of interest.

Selective breeding in coffee trees is a time-consuming process, and the development of a new variety can take as long as 20 years of research. However, the use of alternative methods, such as gene editing, should be considered in the future, as this

will reduce the effort and time required in developing stress-tolerant varieties. Molecular breeding for abiotic stress tolerance is particularly important for the exploitation and conservation of untapped genetic resources. Wild accessions of *C. arabica* and *C. canephora* are important, as they may possess stress tolerance genes. In addition to *C. canephora* and *C. arabica*, several closely related species have been cultivated in the past and show useful traits against abiotic stresses. For example, *C. racemosa*, which originated from East Africa, was resistant to drought and cold, and able to grow on sandy soils (Carvalho et al. 2017). *Coffea stenophylla* and *C. affinis*, which originated from West Africa, and cultivated in Upper West Africa at relatively low elevations, are potentially drought-tolerant (Davis et al. 2020). These species will be critical for the improvement and development of stress-tolerant coffee varieties. Unfortunately, several wild genetic resources, mostly originating from sub-Saharan Africa, are at a high risk of extinction due to human activities, and inefficient in situ and ex situ conservation of the species (Davis et al. 2019). Currently, when considerable focus is directed toward addressing food security, it is of utmost importance to conserve the genetic resources of wild coffee species to ensure sustainable coffee production in the future.

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References

Acidri R, Sawai Y, Sugimoto Y, Handa T, Sasagawa D et al (2020) Exogenous kinetin promove the nonenzymatic antioxidant system and photosynthetic activity of coffee (*Coffea arabica* L.) plants under cold stress conditions. Plants 9:281

Aglawe SB, Barbadikar KM, Mangrauthia SK, Madhav MS (2018) New breeding technique "genome editing" for crop improvement: applications, potentials and challenges. 3 Biotechnology 8:336

Albuquerque EVS, Cunha WG, Barbosa AEAD, Costa PM, Texeira JB et al (2009) Transgenic coffee fruits from *Coffea arabica* genetically modified by bombardment. In Vitro Cell Dev Biol Plant 45:532–539

Alkimim ER, Caixeta ET, Sousa TV, Resende MDV, Silva FL et al (2020) Selective efficiency of genome-wide selection in *Coffea canephora* breeding. Tree Genet Genom 16:41

Alves GSC, Torres LF, Déchamp E, Breitler JC, Joët T et al (2017) Differential fine tuning of gene expression regulation in coffee leaves by *CcDREB1D* promoter haplotypes under water stress. J Exp Bot 68:3017–3031

Alves GSC, Torres LF, de Aquino SO, Reichel T, Freire LP et al (2018) Nucleotide diversity of the coding and promoter regions of *DREB1D*, a candidate gene for drought tolerance in *Coffea* species. Trop Plant Biol 11:31–48

Aria M, Cuccurullo C (2017) Bibliometrix: an R-tool for comprehensive science mapping analysis. J Informet 11:959–975

Avila RT, Almeida WL, Costa LC, Machado KLG, Barbosa ML et al (2020a) Elevated air [CO_2] improves photosynthetic performance and alters biomass accumulation and partitioning in drought-stressed coffee plants. Environ Exp Bot 177:104137

Avila RT, Cardoso AA, Almeida WL (2020b) Coffee plants respond to drought and elevated [CO_2] through changes in stomatal function, plant hydraulic conductance, and aquaporin expression. Environ Exp Bot 177:104148

Barbosa AEAD, Albuquerque EVS, Silva MCM, Souza DSL, Oliveira-Neto OB et al (2010) α-Amylase inhibitor-1 gene from *Phaseolus vulgaris* expressed in *Coffea arabica* plants inhibits α-amylases from the coffee berry borer pest. BMC Biotechnol 10:1–8

Batista-Santos P, Lidon FC, Fortunato A, Leitão AE, Lopes E et al (2011) The impact of cold on photosynthesis in genotypes of *Coffea* spp.—photosystem sensitivity, photoprotective mechanisms and gene expression. J Plant Physiol 168:792–806

Baulcombe DC, Dean C (2014) Epigenetic regulation in plant responses to the environment. Cold Spring Harb Perspect Biol 6:a019471

Bawin Y, Ruttink T, Staelens A, Haegeman A, Stoffelen P et al (2020) Phylogenomic analysis clarifies the evolutionary origin of *Coffea arabica* L. bioRxiv. https://doi.org/10.1101/2020.03.22.002337

Bennett MD, Leitch IJ (2011) Nuclear DNA amounts in angiosperms: targets, trends and tomorrow. Ann Bot 107:467–590

Berthaud J (1986) Les ressources genetiques pour l'amelioration des cafeires africains diploides. Orstom, Paris

Bewg WP, Ci D, Tsai C-J (2018) Genome editing in trees: from multiple repair pathways to long-term stability. Front Plant Sci 9:1732

Bibi F, Barozai MYK, Din M (2017) Bioinformatics profiling and characterization of potential microRNAs and their targets in the genus *Coffea*. Turk J Agric For 41:191–200

Brasil (2018) Instrução Normativa Nº 16, DE 10 de Julho de 2018. Brasília, DF. http://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/29493718/do1-2018-07-11-instrucao-normativa-n-16-de-10-de-julho-de-2018-29493696

Breitler JC, Dechamp E, Campa C, Rodrigues LAZ, Guyot R et al (2018) CRISPR/Cas9-mediated efficient targeted mutagenesis has the potential to accelerate the domestication of *Coffea canephora*. Plant Cell Tiss Org Cult 134:383–394

Bunn C, Läderach P, Rivera OO, Kirschke D (2015) A bitter cup: Climate change profile of global production of *Arabica* and Robusta coffee. Clim Change 129:89–101

Caixeta ET, Pestana KN, Pestana RKN (2015) Melhoramento do cafeeiro: ênfase na aplicação dos marcadores moleculares. In: Garcia GO, Reis EF, Lima JSS, Xaxier AC, Rodrigues WN (eds) Tópicos Especiais em produção vegetal V, Alegre, Brasil, pp 154–179

Campos NA, Panis B, Carpentier SC (2017) Somatic embryogenesis in coffee: the evolution of biotechnology and the integration of omics technologies offer great opportunities. Front Plant Sci 8:1460

Camargo MBPD (2010) The impact of climatic variability and climate change on arabica coffee crop in Brazil. Bragantia, 69:239–247

Carelli ML, Fahl JI, Ramalho JC (2006) Aspects of nitrogen metabolism in coffee plants. Theor Exp Plant Physiol (ex-Braz J Plant Physiol) 18(1):9–21

Carneiro FA, De Aquino SO, Mattos NG, Valeriano JC, Carneiro WWJ et al (2019) Desenvolvimento e validação de uma plataforma de genotipagem em larga escala para *Coffea canephora*. In: X Simpósio de Pesquisa dos Cafés do Brasil, Vitória, Brasil

Carvalho FG, Sera GH, Andreazi E, Sera T, Fonseca ICB et al (2017) Drought tolerance in seedlings of coffee genotypes carrying genes of different species. Coffee Sci 12:156–163

Cenci A, Combes MC, Lashermes P (2012) Genome evolution in diploid and tetraploid *Coffea* species as revealed by comparative analysis of orthologous genome segments. Plant Mol Biol 78:135–145

Charr JC, Garavito A, Guyeux C, Crouzillat D, Descombes P et al (2020) Complex evolutionary history of coffees revealed by full plastid genomes and 28,800 nuclear SNP analyses, with particular emphasis on *Coffea canephora* (Robusta coffee). Mol Phylogenet Evol 151:106906

Charrier A, Berthaud J (1985) Botanical classification of coffee. In: Clifforf MN, Willson KC (eds) Coffee: botany, biochemistry, and production of beans and beverage. Croom Herm, Westport, London, pp 13–47

Chaves SS, Fernandes-Brum CN, Silva GFF, Ferrara-Barbosa BC, Paiva LV et al (2015) New insights on *Coffea* miRNAs: features and evolutionary conservation. Appl Biochem Biotechnol 177:879–908

Chen X, Zhou DX (2013) Rice epigenomics and epigenetics: challenges and opportunities. Curr Opin Plant Biol 16:164–169

Chen H, Hu B, Zhao L, Shi D, She Z et al (2019) Differential expression analysis of reference genes in pineapple (*Ananas comosus* L.) during reproductive development and response to abiotic stress, hormonal stimuli. Trop Plant Biol 12:67–77

Clarindo WR, Carvalho CR (2009) Comparison of the *Coffea canephora* and *Coffea arabica* karyotype based on chromosomal DNA content. Plant Cell Rep 28:73–81

Clarindo WR, Carvalho CR, Caixeta ET, Koehler AD (2013) Following the track of "Híbrido de Timor" origin by cytogenetic and flow cytometry approaches. Genet Resour Crop Evol 60:2253–2259

Coffee Genome Hub (2020) *Coffea canephora* genoma—gene predictions. https://coffee-genome.org/coffeacanephora. Acessed 29 Oct 2020

Cruz F, Kalaoun S, Nobile P, Colombo C, Almeida J et al (2009) Evaluation of coffee reference genes for relative expression studies by quantitative real-time RT-PCR. Mol Breed 23:607–616

Cubry P, Musoli P, Legnaté H, Pot D, Bellis F et al (2008) Diversity in coffee assessed with SSR markers: structure of the genus *Coffea* and perspectives for breeding. Genome 51:50–63

DaMatta FM, Ramalho JDC (2006) Impacts of drought and temperature stress on coffee. Braz J Plant Physiol 18:55–81

DaMatta FM, Ronchi CP, Maestri M, Barros RS (2010) Coffee: environment and crop physiology. In: DaMatta FM (ed) Ecophysiology of tropical tree crops. Nova Science Publishers, New York, pp 181–216

DaMatta FM, Rahn E, Läderach P, Ghini R, Ramalho JC (2019) Why could the coffee crop endure climate change and global warming to a greater extent than previously estimated? Clim Change 152(1):167–178

Daniell H, Lin CS, Yu M, Chang WJ (2016) Chloroplast genomes: diversity, evolution, and applications in genetic engineering. Genome Biol 17:134

Dankowska A, Domagała A, Kowalewski W (2017) Quantification of *Coffea arabica* and *Coffea canephora* var. robusta concentration in blends by means of synchronous fluorescence and UV-Vis spectroscopies. Talanta 172:215–220

Davis AP, Tosh J, Ruch N, Fay MF (2011) Growing coffee: *Psilanthus* (Rubiaceae) subsumed on the basis of molecular and morphological data implications for the size, morphology, distribution and evolutionary history of *Coffea*. Bot J Linn Soc 167:357–377

Davis AP, Chadburn H, Moat J, O'Sullivan R, Hargreaves S, Lughadha EN (2019) High extinction risk for wild coffee species and implications for coffee sector sustainability. Sci Adv 5:eaav3473

Davis AP, Gargiulo R, Fay MF, Sarmu D, Haggar J (2020) Lost and found: *Coffea stenophylla* and *C. affinis*, the forgotten coffee crop species of West Africa. Front Plant Sci 11:616

De Aquino SO, Carneiro FA, Rêgo ECS, Alves GSC, Andrade AC et al (2018) Functional analysis of different promoter haplotypes of the coffee (*Coffea canephora*) *CcDREB1D* gene through genetic transformation of *Nicotiana tabacum*. Plant Cell Tiss Org Cult 132:279–294

De Carvalho K, Bespalhok Filho JC, Dos Santos TB, Souza SGH, Vieira LGE et al (2013) Nitrogen starvation, salt and heat stress in coffee (*Coffea arabica* L.): identification and validation of new genes for qPCR normalization. Mol Biotechnol 53:315–325

De Carvalho K, Petkowicz CLO, Nagashima GT, Bespalhok Filho JC, Vieira LGE et al (2014) Homeologous genes involved in mannitol synthesis reveal unequal contributions in response to abiotic stress in *Coffea arabica*. Mol Genet Genom 289:951–963

Demirel U, Morris WL, Ducreux LJ, Yavuz C, Asim A et al (2020) Physiological, biochemical, and transcriptional responses to single and combined abiotic stress in stress-tolerant and stress-sensitive potato genotypes. Front Plant Sci 11:169

Denoeud F, Carretero-Paulet L, Dereeper A, Droc G, Guyot R et al (2014) The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. Science 345:1181–1184

Dereeper A, Bocs S, Rouard M, Guignon V, Ravel S et al (2015) The coffee genome hub: a resource for coffee genomes. Nucleic Acids Res 43:1028–1035

Van Deynze A, Hulse-Kemp Amanda C, Dario, Medrano JF (2017) Update on sequencing of the *Coffea arabica* variety, Geisha. In: Plant and animal genome conference, San Diego, California, pp 12–18

Dong X, Jiang Y, Yang Y, Xiao Z, Bai X et al (2019) Identification and expression analysis of the NAC gene family in *Coffea canephora*. Agronomy 9:1–17

Dos Santos TB, Vieira LGE (2020) Involvement of the galactinol synthase gene in abiotic and biotic stress responses: a review on current knowledge. Plant Gene 24:100258

Dos Santos TB, Budzinski IGF, Marur CJ, Petkowicz CLO, Pereira LFP et al (2011) Expression of three galactinol synthase isoforms in *Coffea arabica* L. and accumulation of raffinose and stachyose in response to abiotic stresses. Plant Physiol Biochem 49:441–448

Dos Santos TBD, Lima RBD, Nagashima GT, Petkowicz CLO, Carpentieri-Pípolo V et al (2015) Galactinol synthase transcriptional profile in two genotypes of *Coffea canephora* with contrasting tolerance to drought. Genet Mol Biol 38:182–190

Dos Santos TB, Soares JD, Lima JE, Silva JC, Ivamoto ST et al (2019) An integrated analysis of mRNA and sRNA transcriptional profiles in *Coffea arabica* L. roots: insights on nitrogen starvation responses. Funct Integr Genom 19:151–169

Dubberstein D, Lidon FC, Rodrigues AP, Semedo JN, Marques I et al (2020) Resilient and sensitive key points of the photosynthetic machinery of *Coffea* spp. to the single and superimposed exposure to severe drought and heat stresses. Front Plant Sci 11:1049

Dussert S, Lashermes P, Anthony F, Montagnon C, Berthaud J, Noirot M, Hamon S (1999) *Coffea Canephora*. Diversité génétique des plantes tropicales cultivées, CIRAD, p 175

Etienne H, Breton D, Breitler J-C, Bertrand B, Déchamp E et al (2018) Coffee somatic embryogenesis: how did research, experience gained and innovations promote the commercial propagation of elite clones from the two cultivated species? Front Plant Sci 9:1630

Ferrão RG, Fonseca AFA, Silveira JSM, Ferrão MAG, Bragança SM (2000) EMCAPA 8141—Robustão Capixaba, variedade clonal de café conilon tolerante à seca, desenvolvida para o estado do Espírito Santo. Ceres 273:555–560

Ferrão RG, Moreira SO, Ferrão MAG, Riva EM, Arantes LO et al (2016) Genética e melhoramento: desenvolvimento e recomendação de cultivares com tolerância à seca para o Espírito Santo. Incaper em Rev 6:51–71

Ferrão RG, De Muner LH, Da Fonseca AFA, Ferrão M (2019) Conilon coffee, 3rd edn. Vitória, Brazil, pp 1–974

Fleta-Soriano E, Munné-Bosch S (2016) Stress memory and the inevitable effects of drought: a physiological perspective. Front Plant Sci 7:1–6

Fortunato A, Lidon FC, Batista-Santos P, Leitão AE, Pais IP et al (2010) Biochemical and molecular characterization of the antioxidative system of *Coffea* sp. under cold conditions in genotypes with contrasting tolerance. J Plant Physiol 167(5):333–342

Galle A, Florez-Sarasa I, Aououad HE, Flexas J (2011) The Mediterranean evergreen Quercus ilex and the semi-deciduous *Cistus albidus* differ in their leaf gas exchange regulation and acclimation to repeated drought and re-watering cycles. J Exp Bot 62:5207–5216

Gallusci P, Dai Z, Génard M, Gauffretau A, Leblanc-Fournier N et al (2017) Epigenetics for plant improvement: current knowledge and modeling avenues. Trends Plant Sci 22:610–623

Garavito A, Montagnon C, Guyot R, Bertrand B (2016) Identification by the DArTseq method of the genetic origin of the *Coffea canephora* cultivated in Vietnam and Mexico. BMC Plant Biol 16:242–253

Garcia V, Ribas AF, Vieira LGE, dos Santos TB (2018) *In silico* analysis of the Dof transcription factor family in *Coffea canephora.* Coll Agrariae 14:99–111

Gimase JM, Thagana WM, Omondi CO, Cheserek JJ, Gichimu BM et al (2020) Genome-wide association study identify the genetic loci conferring resistance to Coffee Berry disease (*Colletotrichum kahawae*) in *Coffea arabica* var. Rume. Euphytica 46:47

Gomez C, Dussert S, Hamon P, Hamon S, de Kochko A, Poncet V, (2009) Current genetic differentiation of *Coffea canephora* Pierre ex A. Froehn in the Guineo-Congolian African zone: cumulative impact of ancient climatic changes and recent human activities. BMC Evol Biol 9:167

González L, González-Vilar M (2001) Determination of relative water content. In: Roger M (ed) Handbook of plant ecophysiology techniques. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 207–212

Goulao LF, Fortunato AS, Ramalho JC (2012) Selection of reference genes for normalizing quantitative real-time PCR gene expression data with multiple variables in *Coffea* spp. Plant Mol Biol Rep 30:741–759

Guedes FAF, Nobres P, Rodrigues Ferreira DC, Menezes-Silva PE, Ribeiro-Alves M et al (2018) Transcriptional memory contributes to drought tolerance in coffee (*Coffea canephora*) plants. Environ Exp Bot 147:220–233

Guénin S, Mauriat M, Pelloux J, Van Wuytswinkel O, Bellini C, Gutierrez L (2009) Normalization of qRT-PCR data: the necessity of adopting a systematic, experimental conditions-specific, validation of references. J Exp Bot 60:487–493

Guyeux C, Charr JC, Tran HTM, Furtado A, Henry RJ, Crouzillat D, Guyot R, Hamon P (2019) Evaluation of chloroplast genome annotation tools and application to analysis of the evolution of coffee species. PLoS One 14(6):e0216347

Guyot R, Hamon P, Couturon E, Rakotomalala JJ, Raharimalala NE et al (2020) WCSdb: a database of Wild *Coffea* species. (Version V1). Zenodo. https://doi.org/10.5281/zenodo.3899717

Haile M, Kang WH (2018) Transcriptome profiling of the coffee (*C. arabica* L.) seedlings under salt stress condition. J Plant Biotechnol 45:45–54

Hauben M, Haesendonckx B, Standaert E, Van Der Kelen K, Azmi A et al (2009) Energy use efficiency is characterized by an epigenetic component that can be directed through artificial selection to increase yield. Proc Natl Acad Sci USA 106:20109–20114

Herrera JC, Villegas AM, Garcia FA, Dereeper A, Combes MC et al (2014) Genomic relationships among different Timor hybrid (*Coffea* L.) accessions as revealed by SNP identification and RNA-seq analysis. In: Castillo LF, Cristancho M, Isaza G, Pinzón A, Corchado Rodríguez JM (eds) Advances in computational biology. Springer, Cham, pp 161–168

Huang L, Wang X, Dong Y, Long Y, Hao C et al (2020) Resequencing 93 accessions of coffee unveils independent and parallel selection during *Coffea* species divergence. Plant Mol Biol 103:51–61

ICO - International Coffee Organization (2020) Global coffee trade. http://www.ico.org/tradestatistics.asp. Accessed Aug 14 2020

Imbach P, Fung E, Hannah L, Navarro-Racines CE, Roubik DW et al (2017) Coupling of pollination services and coffee suitability under climate change. Proc Natl Acad Sci USA 114:10438–10442

Kinoshita T, Seki M (2014) Epigenetic memory for stress response and adaptation in plants. Plant Cell Physiol 55:1859–1863

Lashermes P, Combes MC, Robert J, Trouslot P, D'Hont A et al (1999) Molecular characterisation and origin of the *Coffea arabica* L. genome. Mol Gen Genet 261:259–266

Lashermes P, Andrzejewski S, Bertrand B, Combes MC, Dussert S et al (2000) Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.). Theor Appl Genet 100:139–146

Lemos S, Fonçatti LF, Guyot R, Paschoal AR, Domingues DS (2020) Genome-wide screening and characterization of non-coding RNAs in *Coffea canephora.* Non-Coding RNA 6:39

Long Y, Xia W, Li R, Wang J, Shao M et al (2011) Epigenetic QTL mapping in *Brassica napus.* Genetics 189:1093–1102

Loss-Morais G, Ferreira DC, Margis R, Alves-Ferreira M, Corrêa RL (2014) Identification of novel and conserved microRNAs in *Coffea canephora* and *Coffea arabica*. Genet Mol Biol 37:671–682

Marçal DMS, Avila RT, Quiroga-Rojas LF, de Souza RPB, Gomes Junior CC et al (2021) Elevated [CO_2] benefits coffee growth and photosynthetic performance regardless of light availability. Plant Physiol Biochem 158:524–535

Marie L, Abdallah C, Campa C, Courtel P, Bordeaux M et al (2020) G × E interactions on yield and quality in *Coffea arabica*: new F1 hybrids outperform American cultivars. Euphytica 216:78

Marques I, Fernandes I, David PHC, Paulo OS, Goulão LF et al (2020) Transcriptomic leaf profiling reveals differential responses of the two most traded coffee species to elevated [CO_2]. Int J Mol Sci 21(23):9211

Marraccini P, Vinecky F, Alves GSC, Ramos HJO, Elbelt E et al (2012) Differentially expressed genes and proteins upon drought acclimation in tolerant and sensitive genotypes of *Coffea canephora*. J Exp Bot 63:4191–4212

Martinez HEP, de Souza BP, Caixeta ET, de Carvalho FP, Clemente JM, (2020) Water stress changes nitrate uptake and expression of some nitrogen related genes in coffee-plants (*Coffea arabica* L.). Sci Hort 267:109254

Martins MQ, Rodrigues WP, Fortunato AS, Leitão AE, Rodrigues AP et al (2016) Protective response mechanisms to heat stress in interaction with high [CO_2] conditions in *Coffea* spp. Front Plant Sci 29:947–964

Martins LD, Tomaz MA, Lidon FC, DaMatta FM, Ramalho JC (2014) Combined effects of elevated [CO_2] and high temperature on leaf mineral balance in *Coffea* spp. plants. Clim Change 126(3–4):365–379

Martins MQ, Fortunato AS, Rodrigues WP, Partelli FL, Campostrini E et al (2017) Selection and validation of reference genes for accurate RT-qPCR data normalization in *Coffea* spp. under a climate changes context of interacting elevated [CO_2] and temperature. Front Plant Sci 8:307

Menezes-Silva PE, Sanglard LMPV, Ávila RT, Morais LE, Martins SCV et al (2017) Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. J Exp Bot 68:4309–4322

Merot-L'anthoene V, Tournebize R, Darracq O, Rattina V, Lepelley M et al (2018) Development and evaluation of a genome-wide Coffee 8.5K SNP array and its application for high-density genetic mapping and for investigating the origin of *Coffea arabica* L. Plant Biotechnol J 17:1418–1430

Mhuantong W, Wichadakul D (2009) MicroPC (microPC): a comprehensive resource for predicting and comparing plant microRNAs. BMC Genom 10:366

Moat J, Gole TW, Davis AP (2019) Least concern to endangered: applying climate change projections profoundly influences the extinction risk assessment for wild Arabica coffee. Glob Change Biol 25:390–403

Mofatto LS, de Araújo CF, Vieira NG, Duarte KE, Vidal RO et al (2016) Identification of candidate genes for drought tolerance in coffee by high-throughput sequencing in the shoot apex of different *Coffea arabica* cultivars. BMC Plant Biol 16:94

Montagnon C, Leroy T, Eskes AB (1998) Amélioration variétale de *Coffea canephora*; 1: Criteres et methodes de sélection. Mejora varietal de *Coffea canephora*, 1: Criterios y métodos de selecciónVarietal improvement of *Coffea canephora*, 1: Criteria and breeding methods. Plantations Recherche Développement (Francia) 5:18–33

Montagnon C, Cubry P, Leroy T (2012) Coffee *Coffea canephora* Pierre genetic improvement: acquired knowledge, strategies and perspectives. Cahiers Agric 21:143–153

Mueller L, Strickler S, Domingues D, Pereira L, Andrade A et al (2015) Towards a better understanding of the *Coffea arabica* genome structure. In: Embrapa Café-Artigo em anais de congresso (ALICE). International Conference on Coffee Science, Udine, Cogito

Musoli P, Cubry P, Aluka P, Billot C, Dufour M et al (2009) Genetic differentiation of wild and cultivated populations: diversity of *Coffea canephora* Pierre in Uganda. Genome 52:634–646

NCBI - National Center for Biotechnology Information (2020) US, National Library of Medicine. Accessed 01 Sept 2020

Nobres P, Patreze CM, Waltenberg FP, Correa MF, Tavano ECR et al (2016) Characterization of the promoter of the homeobox gene *CaHB12* in *Coffea arabica.* Trop Plant Biol 9:50–62

Noirot M, Poncet V, Barre P, Hamon P, Hamon S, Kochko A (2003) Genome size variations in diploid African *Coffea* species. Ann Bot 92:709–714

Noman A, Fahad S, Aqeel M, Ali U, Anwar S et al (2017) miRNAs: major modulators for crop growth and development under abiotic stresses. Biotechnol Lett 39:685–700

Nowak MD, Davis AP, Anthony F, Yoder AD (2011) Expression and trans-specific polymorphism of self-incompatibility RNases in *Coffea* (Rubiaceae). PLoS One 6(6):e21019

Oliveira RR, Ribeiro THC, Cardon CH, Fedenia L, Maia VA et al (2020) Elevated temperatures impose transcriptional constraints and elicit intraspecific differences between coffee genotypes. Front Plant Sci 11:1113

Osakabe Y, Kajitab S, Osakabea K (2011) Genetic engineering of woody plants: current and future targets in a stressful environment. Physiol Planta 142:105–117

Ovalle-Rivera O, Läderach P, Bunn C, Obersteiner M, Schroth G (2015) Projected shifts in *Coffea arabica* suitability among major global producing regions due to climate change. PLoS One 10:e0124155

Park J, Xi H, Kim Y, Heo KI, Nho M et al (2019) The complete chloroplast genome of cold hardiness individual of *Coffea arabica* L. (Rubiaceae). Mitochon DNA Part B 4:1083–1084

Parsell DA, Lindquist S (1993) The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu Rev Genet 27:437–496

Partelli FL, Vieira HD, Rodrigues APD, Pais I, Campostrini E et al (2010) Cold induced changes on sugar contents and respiratory enzyme activities in Coffee genotypes. Ciência Rural 40(4):781–786

Pérez-Pascual D, Jiménez-Guillen D, Villanueva-Alonzo H, Souza-Pereira R, Godoy-Hernández G et al (2018) Ectopic expression of the *Coffea canephora* SERK1 homolog-induced differential transcription of genes involved in auxin metabolism and in the developmental control of embryogenesis. Physiol Plant 163:530–551

Prakash NS, Muniswamy B, Hanumantha BT, Sreenath HL, Sundaresha KD et al (2011) Marker assisted selection and breeding for leaf rust resistance in coffee (*Coffea arabica* L.) some recent leads. Indian J Genet P Br 71:185–189

Rakocevic M, Batista ER, Pazianotto RAA, Scholz MBS, Souza GAR et al (2021) Leaf gas exchange and bean quality fluctuations over the whole-canopy vertical profile of arabic coffee cultivated under elevated CO_2. Funct Plant Biol (in Press). https://doi.org/10.1071/FP20298

Ramalho JC, Pais IP, Leitão AE, Guerra M, Reboredo FH et al (2018a) Can elevated air [CO_2] conditions mitigate the predicted warming impact on the quality of coffee bean? Front Plant Sci 9:287

Ramalho JC, Rodrigues AP, Lidon FC, Marques LMC, Leitão AE et al (2018b) Stress cross-response of the antioxidative system promoted by superimposed drought and cold conditions in *Coffea* spp. PLoS One 13(6):e0198694

Ramalho JC, Campos PS, Teixeira M, Nunes MA (1998) Nitrogen dependent changes in antioxidant systems and in fatty acid composition of chloroplast membranes from *Coffea arabica* L. plants submitted to high irradiance. Plant Sci 135(2):115–124

Ramalho JC, Pons T, Groeneveld H, Azinheira HG, Nunes MA, (2000) Photosynthetic acclimation to high light conditions in mature leaves of *Coffea arabica* L.: role of xanthophylls, quenching mechanisms and nitrogen nutrition. Funct Plant Biol (ex- Aust J Plant Physiol) 27:43–51

Ramalho JC, Fortunato AS, Goulao LF, Lidon FC (2013) Cold-induced changes in mineral content in leaves of *Coffea* spp. Identification of descriptors for tolerance assessment. Biol Plant 57(3):495–506

Ramalho JC, DaMatta FM, Rodrigues AP, Scotti-Campos P, Pais I et al (2014) Cold impact and acclimation response of *Coffea* spp. plants. Theor Exp Plant Physiol 26(1):5–18

Rao S, Balyan S, Jha S, Mathur S (2020) Novel insights into expansion and functional diversification of MIR169 family in tomato. Planta 25:55

Ribas AF, Dechamp E, Champion A, Bertrand B, Combes MC et al (2011) *Agrobacterium*-mediated genetic transformation of *Coffea arabica* (L.) is greatly enhanced by using established embryogenic callus cultures. BMC Plant Biol 11:92

Rijo L (1974) Observações cariológicas no cafeeiro "Híbrido de Timor." Port Acta Biol 8:157–168

Rodrigues WP, Martins MQ, Fortunato AS, Rodrigues AP, Semedo JN et al (2016) Long-term elevated air [CO_2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *C. canephora* species. Glob Change Biol 22:415–431

Sade N, Moshelion M (2017) Plant aquaporins and abiotic stress. In: Chaumont F, Tyerman SD (eds) Plant aquaporins. Springer, Cham, pp 185–206

Sant'Ana GC, Pereira LFP, Pot D, Ivamoto ST, Domingues DS et al (2018) Genome-wide association study reveals candidate genes influencing lipids and diterpenes contents in *Coffea arabica* L. Sci Rep 8:1–12

Scalabrin S, Toniutti L, Di Gaspero G, Scaglione D, Magris G et al (2020) A single polyploidization event at the origin of the tetraploid genome of *Coffea arabica* is responsible for the extremely low genetic variation in wild and cultivated germplasm. Sci Rep 10:1–13

Scotti-Campos P, Pais IP, Ribeiro-Barros AI, Martins LD, Tomaz MA et al (2019) Lipid profile adjustments may contribute to warming acclimation and to heat impact mitigation by elevated [CO_2] in *Coffea* spp. Environ Exp Bot 167:103856

Sengupta S, Mukherjee S, Basak P, Majumder AL (2015) Significance of galactinol and raffinose family oligosaccharide synthesis in plants. Front Plant Sci 6:656

Simkin AJ, Moreau H, Kuntz M, Pagny G, Lin C et al (2008) An investigation of carotenoid biosynthesis in *Coffea canephora* and *Coffea arabica*. J Plant Physiol 165:1087–1106

Soltis DE, Soltis PS, Pires JC, Kovarik A, Tate JA, Mavrodiev E (2004) Recent and recurrent polyploidy in Tragopogon (Asteraceae): cytogenetic, genomic and genetic comparisons. Biol J Linn Soc 82:485–550

Stebbins GL Jr (1947) Types of polyploids: their classification and significance. Adv Genet 1:403–429

Szcześniak MW, Deorowicz S, Gapskiak J, Kaczyński L, Makałowska I et al (2012) miRNEST database: an integrative approach in microRNA search and annotation. Nucleic Acids Res 40:198–204

Tan BC, Joseph LM, Deng WT, Liu L, Li QB et al (2003) Molecular characterization of the *Arabidopsis* 9-*cis* epoxycarotenoid dioxygenase gene family. Plant J 35:44–56

Tang X, Zhang N, Si HJ, Urrea AC (2017) Selection and validation of reference genes for RT-qPCR analysis in potato under abiotic stress. Plant Methods 13:1–8

Thellin O, Zorzi W, Lakaye B, De Borman B, Coumans B et al (1999) Housekeeping genes as internal standards: use and limits. J Biotechnol 75:291–295

Thioune EH, McCarthy J, Gallagher T, Osborne B (2017) A humidity shock leads to rapid, temperature dependent changes in coffee leaf physiology and gene expression. Tree Physiol 37:367–379

Thioune EH, Strickler S, Gallagher T, Charpagne A, Decombes P et al (2020) Temperature impacts the response of *Coffea canephora* to decreasing soil water availability. Trop Plant Biol 13:236–250

Torres LF, Reichel T, Déchamp E, de Aquino SO, Duarte KE et al (2019) Expression of *DREB*-like genes in *Coffea canephora* and *C. arabica* subjected to various types of abiotic stress. Trop Plant Biol 12:98–116

Valencia-Lozano E, Cabrera-Ponce JL, Gómez-Lim MA, Ibarra JE (2019) Development of an efficient protocol to obtain transgenic coffee, *Coffea arabica* L., expressing the Cry10Aa toxin of *Bacillus thuringiensis*. Int J Mol Sci 20:5334

Van der Vossen H, Bertrand B, Charrier A (2015) Next generation variety development for sustainable production of arabica coffee (*Coffea arabica* L.): a review. Euphytica 204:243–256

Vats S, Kumawat S, Kumar V, Patil GB, Joshi T et al (2019) Genome editing in plants: exploration of technological advancements and challenges. Cells 8(11):1386

Vidal RO, Mondego JMC, Pot D, Ambrósio AB, Andrade AC et al (2010) A high-throughput data mining of single nucleotide polymorphisms in *Coffea* species expressed sequence tags suggests differential homeologous gene expression in the allotetraploid *Coffea arabica.* Plant Physiol 154:1053–1066

Vinecky F, Davrieux F, Mera AC, Alves GSC, Lavagnini G et al (2017) Controlled irrigation and nitrogen, phosphorous and potassium fertilization affect the biochemical composition and quality of Arabica coffee beans. J Agric Sci 155:902–918

Wu D, Bi C, Wang X, Xu Y, Ye Q et al (2017) The complete chloroplast genome sequence of an economic plant *Coffea canephora.* Mitochon DNA Part B 2:483–485

Yaguinuma DH, dos Santos TB, de Souza SGH, Vieira LGE, Ribas AF (2021) Genome-wide identification, evolution, and expression profile of aquaporin genes in *Coffea canephora* in response to water stress. Plant Mol Biol Rep 39:146–162

Yang Q, Chen Q, Zhu Y, Li T (2018) Identification of *MdD* of genes in apple and analysis of their response to biotic or abiotic stress. Funct Plant Biol 45:528–541

Yu QY, Guyot R, de Kochko A, Byers A, Navajas-Pérez R et al (2011) Micro-collinearity and genome evolution in the vicinity of an ethylene receptor gene of cultivated diploid and allotetraploid coffee species (*Coffea*). Plant J 67:305–317

Yuyama PM, Júnior OR, Ivamoto ST, Domingues DS, Carazzolle MF et al (2016) Transcriptome analysis in *Coffea eugenioides*, an Arabica coffee ancestor, reveals differentially expressed genes in leaves and fruits. Mol Genet Genom 291:323–336

Zambolim L (2016) Current status and management of coffee leaf rust in Brazil. Trop Plant Pathol 41:1–8

Zeng A, Xu Y, Song L, Li J, Yan J (2020) Validation of suitable reference genes for qRT-PCR in cabbage (*Brassica oleracea* L.) under different abiotic stress experimental conditions. J Plant Biochem Biotechnol 4:1–12

Zheng JY, Liu F, Zhu CH, Li X, Dai X et al (2019) Identification, expression, alternative splicing and functional analysis of pepper WRKY gene family in response to biotic and abiotic stresses. PLoS One 14:e0219775

Zhou Y, Tao J, Ahammed GJ, Li J, Yang Y (2019) Genome-wide identification and expression analysis of aquaporin gene family related to abiotic stress in watermelon. Genome 62:643–656

Chapter 4
Resilient Cotton for Abiotic Stresses: Realizing Genetic Gains Through Translational Genomics

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Abstract Production of cotton, the most preferred natural fiber, is often limited by abiotic stresses especially water and salinity stresses. Evolving elite cotton cultivars with improved abiotic stress resistance characteristics using conventional breeding approaches has met with limited success due to the complexity of the resistance mechanisms. Recent advances in omics tools provide great opportunity to study these resistance mechanisms in deeper perspective and offer useful information to proceed further. In order to speed up the process to realize accelerated breeding gain in cotton resilient to abiotic stress resistance, several technologies have to be employed in cotton breeding program such as sequence-based trait mapping and breeding using low-cost genotyping assays, speed breeding for rapid generation advancement and robust screening procedures for abiotic stress resistance, haplotype-based breeding and incorporating the traits that enhance the operational efficiency including mechanization in boll harvesting. This chapter provides a comprehensive view on progresses made in this direction in cotton and way forward to accelerate the procedure that help to realize the genetic gains through omics tools.

Keywords Cotton · Drought · Salinity · Breeding Design · Omics · Panomics · Metabolomics · Fiber Quality Traits

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4.1 Cotton Lint: Demand and Production Constraints

4.1.1 Production, Productivity and Demand

Cotton (*Gossypium* spp.,) crop offers lint and seeds which are used as fiber, edible oil and animal feed. Owing to its versatility, performance, and comfort, cotton fiber is the most widely used natural fiber in textiles and more than one third of the globally manufactured total fibers is from cotton. Owing to its strong, water absorbent and comfortable properties cotton fibers are preferred to create different types of fabrics/textiles and surgical products. It has been reported that 64%of the global cotton products are used in apparel makings, 28% used for home furnishings, and 8% for industrial applications (https://www.mordorintelligence.com/industry-reports/cotton-market).

Further, a cholesterol-free oil is also obtained from sequencing of cottonseeds, which is largely used as an ingredient in cooking, cosmetics, pharmaceuticals, personal care products, plastics and rubber. Cotton seeds are further used to produce a high-protein meal and they are widely used to feed livestock and poultry. Thus, cotton is universally applauded as "the most widespread profitable non-food crop in the world".

It is anticipated that worldwide cotton production may grow at a slower pace than consumption (Fig. 4.1), due to the decreased price levels and probable release of global stocks gathered between 2010 and 2014 (OECD/FAO 2018). It has been observed during the last couple of years that worldwide cotton area is slightly lower than the global average area. However, global cotton yields are likely to increase gradually as production steadily shifts from comparatively high yielding countries (such as China) to relatively low-yielding ones (such as South Asian and West African countries).

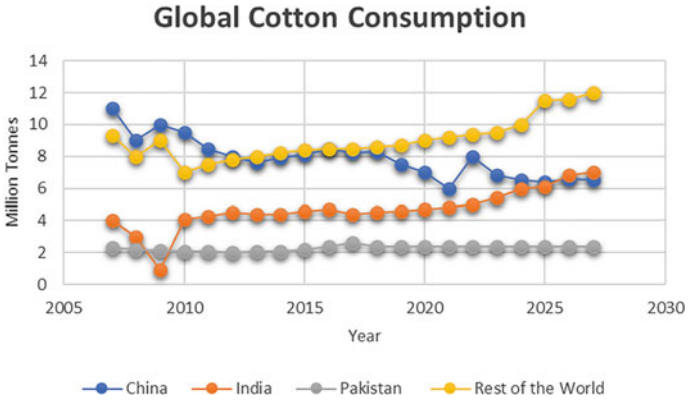

Fig. 4.1 Global trends in consumption of cotton lint

As the demand for cotton lint is increasing steadily and it is invariably realized that cotton productivity must be increased to meet out the future requirement as the area for cotton production has shown a decreasing trend. For example, in India, the cotton area is reduced to 12.95 million hectares in 2020–2021 from 13.37 million hectares (https://cotcorp.org.in/national_cotton.aspx). Similarly, though Australia is acclaimed as one of the most efficient cotton industries in the world in terms of water use, recent climatic vagaries significantly reduced its cotton area.

4.1.2 Factors Limiting Productivity

Though increased yield potential is anticipated in global cotton production, there is a substantial yield gap in reality. For instance, India has the largest area under cotton (12.95 million hectares), but its cotton productivity is just 486 kg per hectare, much lower for the vast area it occupies (https://cotcorp.org.in/national_cotton.aspx). Similar trend is also observed in Pakistan, Uzbekistan and Turkey. The main reason owing to this low productivity in India and elsewhere is cotton is being cultivated in largely small holdings under rainfed conditions. Yield in these conditions is recurrently reduced due to struggle among crops for land and inputs and lack of adequate weed and pest control (Hamdy et al. 1994).

Another major hurdle identified in global cotton cultivation is more than 55% of sowing is decided totally on onset of monsoon, as cotton is chiefly grown as rainfed crop at least in India, Pakistan, Uzbekistan and African countries. Lack of water seriously disturb field preparation which will lead to meagre germination and deprived plant stand. In the same way, if water scarcity occurs during flowering phase, it harshly affects the lint yield and sometimes lead to nil productions. Another major factor that limits worldwide cotton production is biotic factors: mostly pests and occasionally cotton diseases has also been shown to reduce the overall yield.

4.1.3 Abiotic Stresses and Their Impact on Cotton Productivity

Among the various abiotic stresses (light—deficit or excess, deficiency or excess water content, high or low temperature, ions—deficit or excess), drought and salinity are the most distressing issues in global cotton production. Cotton is the major herbaceous warm-season crop, but its production is hampered by water stress because it is produced worldwide during the summer in arid and semiarid places where water is scarce.

It is estimated that world average cotton yield is 650 kg lint per hectare (FAO Stat http://faostat.fao.org/default.aspx) even though in some countries higher yields were recorded (for example, in Israel, the average country-wide yield was 2020 kg lint per

hectare in 2005 (Saranga et al. 2009). Thus, it is clear that nearly 73% cotton production loss is due to various stresses (such as disease and insect losses are responsible for 4.1% and 2.6% respectively yield reduction by weed competition is 2.6% and yield losses due to inappropriate soils and climates is 63.1%) and such losses is roughly equivalent to those values reported in field crops (Edmeades et al. 2001). Consequently, it can be concluded that abiotic stresses cause destructive changes in cotton that adversely affect its productivity.

Generally, abiotic stresses cruelly confine cotton phenology, particularly they reduce photosynthesis, transpiration rate, stomatal conductance, plant height, weights of roots and shoots (both dry and wet), leaf area index, number of nodes, canopy, root development and finally yield and fiber quality (Loka et al. 2011).Drought or water stress is the chief type of abiotic stresses that affect crop productivity. Under this era of global climate change, drought become major threat crop yield including cotton. Although cotton has moderate salt tolerance, it does not possess water stress tolerance (Penna et al. 1998).

Both drought and salt stresses (with variable intensity and duration) affect molecular, biochemical and physiological processes of the cotton and different levels of severity were noticed at different phenological stages. Although drought condition shows a negative impact on all phases of cotton growth and development, water deficiency is most sensitive to stages such as seedling, flowering, and boll development (Pace et al. 1999), while seed germination and seedling stages are the most sensitive to salt stress (Munns and Tester 2008). Effect of drought and salinity on cotton has been extensively reviewed and it was concluded that the cotton yield and quality are severely harmed when it is subjected to high salt and drought (Abdelraheem et al. 2019).

Hence, evolving an abiotic stress resilient cotton cultivar can improve the lint production under drought and salinity stress as there exists a substantial scope for enhancing the productivity by bridging up the possible yield gap. This requires a deep understanding of cotton genome evolution and expression as well as regulation of genes under varying climatic conditions, which are described in the below subheadings.

4.2 Genome Evolution in Cultivated Cotton

Unraveling the evolution and origin of cotton will afford better insights on abiotic stress resistance development in cotton. The genus *Gossypium* includes approximately 50 diploid ($2n = 26$) and 5 tetraploid ($2n = 4x = 52$) species (Fryxell 1992). All the tetraploid varieties comprise of 'A' and 'D' sub-genomes that have instigated from a common ancestor around 4–11 million years ago and later, about 1–2 million years ago. Thus, all the genes in tetraploid cotton have two or more copies (representing each of the two sub-genomes); besides, although not identical, but they have similar chromosomal orders in both the sub-genomes as that of their diploid ancestors (Wendel1989).

Though both cultivated cotton species (*G. hirsutum* and *G. barbadense*) can be inter- and intra-crossed to one another, their inter crossing with wild tetraploid species *viz*., *G. tomentosum*, *G. darwinii* and *G. mustelinum* resulted into different degrees of sterility and incompatibility. Such crosses often result into unusual interspecific allele combinations which ultimately produce nonrandom patterns of introgression.

Cotton is said to have originated from wild perennial plants adaptable to semi-arid and semitropical climates, which were usually exposed to intermittent drought and temperature extremes (Kohel 1974). Hence, it is likely that *Gossypium* might have adapted to heat, drought and salt stresses. It is true as few of exotic tetraploid cottons have a high tolerance for heat and drought. For example, *G. tomentosum*, prevalent in Hawaii, are basically found in dry, pebbly, or clay coastal plains on the leeward side of main islands except in the Big Island (http://kalama.doe.hawaii.edu/hern95/pt009/Ann/mcc_nativeplants.html). It was also evident that interspecific crossing *G. tomentosum* and *G. hirsutum* produced progeny that were drought tolerant to a high degree (Gotemare and Singh 2004). Similarly, *G. mustelinum* is widespread in extremely dry areas of semiarid region of Northeastern Brazil. Likewise, *G. darwinii* prevalent in the Galapagos islands survives naturally in the challenging environment ofarid islands. Wild *G. barbadense* plants that are grown on the salt affected coasts of Peru, Ecuador and Galapagos Islands (Saranga et al. 2009). Therefore, use of those naturally evolved *Gossypium* wild species would serve as potential donors in breeding program for abiotic stress resistance improvement as they have such inherent properties.

4.3 Gene Pools in Cotton

Importance of assembling cotton gene pools and their value in routine cotton breeding program has already been documented (Kulkarni et al. 2009). Their efforts have clearly demonstrated that diploid species (especially *G. herbaceum* and *G. arboreum*) can be grown in marginal and Asian climates that are prone to drought and they not only survive but produce significant fiber yield under this harsh environment owing to their inherent ability to survive underwater stress. Another notable advantage is that diploid cottons are resistant to sucking pests including hoppers, white flies, thrips, and aphids, as well as the leaf curl virus. Though diploids were cultivated in earlier days, it has been replaced by *G. hirsutum* in several Asian regions due to poor diploid boll characteristics such as short, coarse and weak fiber. However, in traditional cotton-growing areas such as India, Pakistan, China, Bangladesh, and Iran, diploid cotton is still grown.

In order to bring the best of diploid cotton into tetraploid cotton, a sound breeding program is prerequisite. To this end, instead of strict taxonomic placement of species, it would be desirable to classify the gene pool by emphasizing the genetic compatibility (Harlan and de Wet 1971). According to them, it would be useful to sort them into three groups: primary, secondary, and tertiary gene pools (from easy to difficult) by utilizing the reproductive apparatus of the plants to transfer the genes.

Initially, Stewart (1995) assigned the *Gossypium* genomes into distinct gene pools, out of which the primary gene pool comprises of all the allotetraploid species and the secondary and tertiary gene pool includes diploid species by employing their association with the A and D genome progenitors of those *Gossypium* allotetraploids. As a result of this classification, the A and D genomes were grouped with the B and F genomes in the secondary gene pool because their chromosomes are organizationally similar to the A and D genomes. It has also been demonstrated that genomes may show relatively high recombinant frequency if a fertile hybrid can be generated. On the other hand, since C, E, G, and K genomes and its hybrids (which is often difficult to generate) shows low level of genetic recombination, thus they were placed in the tertiary pool.

Irrespective of the benefits of the above classification, it has considered that such classification deviates from Harlan and de Wet's categorization. According to Harlan (1992) the primary gene pool is those crops in which hybrids are normally fertile and has chromosome pairing capacity, gene transfer is easy, and gene segregation is normal. However, the secondary gene pool comprises coenospecies i.e., species/ecotypes that are capable of interbreeding without loss of fertility in the offspring and may participate in the process of recombination (to limited extent) after hybridization. As a result, there is no gene flow across coenospecies, and breeding produces sterile hybrids. Thus, it may be difficult to recover desirable offspring in advanced generations, but it is possible. According to him, the third category, tertiary gene pool, includes species that can be crossed but produce abnormal, deadly, or sterile hybrids. Extreme procedures such as *in vitro* techniques (embryo culture, tissue culture) chromosome doubling, grafting, or the use of bridge species are required for successful transfer of desirable traits. From the above lines, it can be concluded that the whole thing within the *G. hirsutum* species, is in the primary gene pool. Similarly, the secondary gene pool in cotton may comprises tetraploid *Gossypium* species, as these species fits almost perfectly as a coenospecies. For instance, *G. hirsutum* and *G. barbadense* crosses are strong and fertile but such hybrids show segregation distortion towards one parent or genetic break down during segregation in subsequent generations. Also, the gene pool of the diploid cotton species and other distant groups which shows successful sexual reproduction, can be put in the tertiary gene pool since introgression of desirable genes from these species need to be effected with unusual strategies such as chromosome doubling and bridging species. At this point, it should also be noted quaternary gene pool. In general, quaternary gene pool refers to the transfer of precise genes from other organisms such as transfer of genes from the bacteria *Bacillus thuringiensis* in cotton (*Bt* cotton). In some of the literature, for example, Stewart (1995) introduced αω (alpha omega) gene pool to describe those genes that can be found in any organism.

Therefore, it can be concluded that despite of their gene pools classifications, the wide array of *Gossypium* species offers an exciting range of variation in abiotic stress resistance component traits which can be exploited in cotton improvement programs for abiotic stress resistance (Saranga et al. 2009). Hence, the immediate goal in the cotton breeding program would be bring back those desirable alleles from

wild ancestors of *Gossypium* that were lost during domestication but are now useful for increasing productivity in abiotic stress conditions.

4.4 Target Traits for Abiotic Stress Resistance Breeding in Cotton

Roots are the first organs to detect water and salt stress, hence they play an important role in response to abiotic stresses response. Different root architecture traits such as length (longer root systems are better equipped to absorb water from deeper soil), fresh and dry weight, volume, and root density are associated directly and shows tolerance towards various abiotic stress. Usually, plant's tap roots are ten times higher than the plant's height (which usually measured from collar region to the tip of the shoot; Larcher 2003). Despite the fact that initial or mild drought (as well as salinity) boosted root length in cotton (Luo et al. 2016), prolonged or more severe water stress actually reduced root length and fresh and dry weight.

Drought also affects root-to-shoot biomass accumulation ratio and previous investigation shows that root-to-shoot ratio generally increased during stress condition (McMichael and Quisenberry 1991). Thus, shoot length, root and shoots weight, and root-to-shoot biomass ratios would be important features to select cotton genotypes with increased abiotic stress resistance (Basal et al. 2006; Dewi 2011).

Cotton has also shown to have lower photosynthetic rates under water stress due to a combination of stomatal and non-stomatal restrictions. Frequent stomatal closure and elevated leaf temperature during drought usually reduced CO_2absorption and Rubisco inactivation results in decrease of photosynthetic rate (Abdelraheem et al. 2019).

It is also worth to mention here that in the field conditions, other parameters like onset of abiotic stress, growth stage, types of leaves, CO_2 concentrations and abscisic acid concentrations are directly linked with photosynthetic rate. For example, photosynthetic rates may be affected by the time at which it occurs (morning *vs.* afternoon) and leaf age. Particularly, when exposed to high temperature (i.e., >37 °C), early cotton leaves are photosynthetically more resistant to drought and heat stress than mature leaves (Chastain et al. 2016). Furthermore, in both greenhouse and field conditions, it was found that the okra and super okra leaf types can sustain higher leaf and turgor potentials, thus shows lower stomatal conductance values than normal leaf type there by increasing photosynthetic rate under drought condition (Pettigrew 2004). Under dryland conditions similar results were found in okra leaf type cotton plants (Stiller et al. 2005), which indicated that okra leaf morphology can be used as a potential factor of drought tolerant traits to select a breeding line during breeding program. Thus, both above and below ground plant characteristics were found to be good indicators of developing resilient cotton cultivars against abiotic stresses.

4.5 Breeding Designs for Abiotic Stress Resistance Improvement

So far genetic improvement of cotton using classical breeding strategies have been focused mostly on fiber quality and quantity, high yields, seed quality, tolerance towards abiotic and biotic stresses and adaption of cotton where-ever it is grown. However, traditional cotton breeding techniques, explored into the benefits of desired alleles found in grown cotton from the same or distinct *Gossypium* species, because there are no reproductive barriers between parental lines in intraspecific breeding (within tetraploid upland (*G. hirsutum* L.) or pima cotton (*G. barbadense* L.)).

In contrast, interspecific breeding between the above two commonly cultivated cotton species has met with the hybrid breakdown issues in F_2as well in in other advanced generations irrespective of producing heterotic fertile F_1 hybrids. Similar trend has also been noticed in introgressing desirable genes from diploid species to upland cotton. Though several strategies have explored (Stewart 1995) to overcome obstacles in interspecific cotton breeding, such cross combinations have shown with limited success owing to complications in producing F_1 hybrids, chromosomal doubling and chromosome number recovery after repeated backcrossing.

Invariably, the genetic improvement of lint yield remains the top priority in cotton breeding program as evolving resilient cotton with abiotic stress resistance improvement is extremely difficult due to their complex genetic mechanisms. Farmers still rely heavily on multiple and expensive strategies to overcome the abiotic stresses (such as augmenting irrigation systems to mitigate water stress and soil management practices to overcome salinity issues). As a result, there is a requirement for novel traits that may be introduced to evolve resilient cotton cultivar for effective ontrol of the abiotic stresses, and these efforts are both cost effective and sustainable.

4.5.1 Conventional Genetic Improvement Methods

Early breeding efforts in developing high fiber strength cottons made in 1938 by Beasley (1940) by effecting a tri species crossing program (*G. arboreum* L. X *G. thurberi* Tod. X *G. hirsutum* L.) followed by two backcrossing with the *G. hirsutum* parent,' Coker 100.' Another attempt by Kerr (1960) has shown introgression of fiber strength from *G. arboreum* X *G. thurberi*to upland cotton with a modified backcrossing program.

Subsequently thousands of cross combinations and selections were effected elsewhere in the world and several hundreds of cultivars and hybrids were evolved with improved lint yield and fiber quality traits. Majority of the early breeding programs had designed with the aim of developing improved fiber quality traits as extra-long staple cottons as it had high economic value and later the demand for the development of medium staple cottons with high fiber strength had increased due to advancements in spinning tools and technologies.

On the other hand, through literature survey on cotton breeding, a strong negative correlation between yield and quality (either fiber strength or length) was reported which was due to pleiotropy, linkage, or a combination of the two traits. Though it was tough to understand the pleiotropic effects of genes on expressing stronger or longer fibers in cotton, several reports have established linkage as a causative phenomenon for the negative association between yield and fiber quality.

Initially, Chinese upland cotton germplasms were largely from the United States of America (introduced to Yangtze River and Yellow River regions) and the former Soviet Union (introduced to Xinjiang province region). However, with the merging of pedigree and hybrid breeding procedures, extensive cotton breeding operations in China resulted in the expansion of a series of backbone parents adapted to the local environment. Furthermore, Chinese breeders utilized *G. hirsutum* of wild relatives and landraces to develop a large numbers of introgression lines than could be used to transfer desirable traits to commercial cultivars via interspecific hybridization. These efforts greatly expanded the genetic pool of Chinese cotton germplasm, resulting in the emergence of a number of elite lines with greater fiber quality and stress resistance (Zhenglan et al. 2002). It has also been reported that fiber quality of upland cotton cultivars can be improved through mutagenesis and pedigree selection (Zhou et al. 2020).

However, Meredith and Bridge (1971) doubted the frequently used pedigree system of hybridization and selection and they suggested several alternative methods that may break linkages between lint yield and fiber strength and length. These methods include random intermating, diallel selective mattings, modified backcrossing and bulk breeding followed by selection and use of selection indices with the above methods.

Thus, it is generally considered that any breeding method (such as mutagenesis, random intermating, backcrossing and composite-crossing) that gives maximum combinations of genes for yield and fiber quality can be preferred in designing cotton breeding program and selection of promising recombinants in early generations using appropriate stress resistance component traits (see above) is essential to evolve a better abiotic stress resistance cotton cultivar.

4.5.2 Male Sterility System and Hybrids

Seamless development of the reproductive cells is the intricate phenomenon, in almost all the plant species including cotton, and any disturbances during the reproductive phase can cause male sterility (Hedhly et al. 2009). Though male sterility is the challenge in cotton since it reduces as it decreases fiber yield and quality, it can be employed as a simple pollination control method, which is largely recognized in cotton hybrid breeding programs (Zhang et al. 2020).

Usually, cultivated cotton crop is self-pollinated. Consequently, in order to effectively produce hybrids, it is prerequisite to get the male sterile flowers pollinated. In

cotton, typically, male sterility is achieved through the use of mutated genes, environmental stress, cytoplasm from other species, and chemical treatment. However, the expression of such induced genetic sterilities might range from complete sterility caused by a single dominant gene to partial sterility caused by recessive genes. Similarly, the cytoplasmic-genetic sterile strains with cytoplasm from either *G. anomalum* Wawra & Peyr. or *G. arboreum* L. respond differently to genes, cytoplasm and the external environment. Daily maximum temperature that occurs during 15–16 days before anthesis have been found to have greater impact on sterility than any other aspect of the external environment.

In order to produce potential hybrids, A- and B-lines (one for *G. anomalum* cytoplasm, the other for *G. arboreum* cytoplasm) have been produced for pure-breeding sterile strains. Viable F1 hybrids were produced when commercial strains of *G. barbadense* L. was tested with the two A- and B- sterilities (Meyer 1969). It should be emphasized that not all the genes are known to cause partial or complete male sterility in cotton and much are not reported in the literature yet, however, first documentation that assigned a genesymbol to a genetic male sterility in cotton was by Justus and Leinweber (1960). At the homozygous stage, a completely recessive gene (ms_1) produces partial or complete male sterility in upland cotton. Although the progenitor plant was monosomic, its msl msl offspring possessed a normal number of chromosomes. The monosomic condition perhaps allowed sterility expression and helped to identify the recessive gene.

Richmond and Kohel (1961) described a second sterility caused by a single recessive gene (ms2), and cotton lines homozygous for this gene are fully male sterile. The spiny exine of typical cotton pollen is missing from these sterile lines' pollen grains, and the vacuole-like contents indicate a much-reduced amount of protoplasm.

Justus et al. (1963) reported male sterility in cotton is due to ms_3 recessive gene because like ms_1ms_1, homozygous ms_3ms_3 does not always lead to the formation of complete male sterile progenies in upland cotton. It has been reported that larger amounts of sterile anthers have always been found at Stoneville for ms_3ms_3 plants than for ms_1ms_1 plants during flowering phase in the same field on the same date.

In contrast, dominant male sterility gene reported in cotton so far is Ms_4 (Allison and Fisher 1964). Upland cotton lines with *Ms4* developed filaments with poor rudimentary anthers or no anthers at all and consequently, the sterility is complete.

Besides, male sterility has also been identified in cotton lines obtained from *G. armourianum* X *G. hirsutum* derivatives, and it was found to be controlled by a single recessive gene and was unaffected by cytoplasm. In this case, complete male and female sterility has been noticed and it has been shown that meiotic abnormalities might be responsible for such sterility. However, inheritance of this sterility could not be determined as test crosses could not be produced and no gene symbols have been designated for this sterility (Meyer 1969).

In recent decades, several genes that play major roles in the development of male reproductive organs have been identified from database through transcriptomic and proteomic studies of plant species. However, little is known about the molecular machinery of genes regulating male sterility in cotton (Ramadan et al. 2021).

The use of heterosis to develop useful F_1 hybrids has long been an objective of cotton breeders and adequate amounts of heterosis exist in cotton that can translate into increased fiber yields. However, chiefly due to the complexity of producing F_1 seeds, use of heterosis in developing resilient cotton for abiotic stress resistance has been limited (Meredith Jr 1990) and there is little effort made towards this end.

4.5.3 Limitations of Traditional Methods

Historically traditional plant breeding strategies combined with wide hybridization have evidently been played key role in evolving superior crop cultivars (Jauhar 2006). However, these methods, on the other hand are time consuming, costly and labour intensive. Also, it may take ten to transfer a trait from a donor species into a crop cultivar using conventional breeding techniques since, hybridization is one of the most effective methods for the introduction of alien gene of interest in crop cultivars, with several limitations. It mostly leads in the transmission of unwanted alien chromosomes and opposing genetic relations, with the majority of offspring being sterile. Further, it has long been realized as a herculean task during genetic improvement of abiotic stress resistance in cotton owing to the limitations such as lack of simple and affordable abiotic stress resistance target trait, complex interactions of abiotic stresses and extremely heterogenous environments in which cotton is being cultivated (therefore, different strategies and cultivars must be used for each target population of environment). This leads to look for other efficient means of gene transfer that need to be investigated.

4.6 Genomic Tools for Cotton Resilient to Abiotic Stresses

There is an increasing interest in using the advanced technologies of genomics to identify vital gene or genetic loci controlling abiotic stress tolerance in cotton and as tools to screen allelic variation in the cotton gene pools. Outcomes of such cotton genomics studies can be disseminated through conventional breeding. Alternatively, in some cases, a genetic transformation technique may be a more rapid option.

Despite the fact that cotton has varying degrees of tolerance to various abiotic stresses, little is known about the molecular basis for abiotic stress tolerance in *Gossypium* and there is still abundant opportunity for their improvement. The following sections provides strategies and examples to answer the basic question: Will breakthrough in genomics enable researchers to better understand stress tolerance in *Gossypium* and lead to increased rates of genetic gain for abiotic stress tolerance?

4.6.1 Contribution of 'Omics Tools

Multiple 'omics tools (including transcriptome, proteomics, metabolomics and noncoding sequencing) have recently emerged as an effective-strategies to study genetic contribution to agriculturally important traits along with its structure and evolutionary details of the genome. Such efforts have repeatedly shown that the molecular responses of plants to abiotic stress are complex process. They are mainly regulated by the transcriptional activity of stress-related genes, and many of such genes are induced under stress conditions (Baruah et al. 2021).

The enzymes responsible for the manufacture of numerous osmo protectants are among the products of stress-inducible genes that protect against abiotic stresses. Besides, systems biology and virtual experiments (Gutiérrez et al. 2005) may also offer avenues for visualizing and understanding how cotton respond to abiotic stress and avoid such stresses.

Efforts have already undergone to integrate the transcriptome analysis and metabolite profiling with a aim to comprehensively unravel the rationale behind the abiotic stress tolerance in other field crops, though it has not yet started in cotton. The development of metabolite sensors and databases that combine metabolite and transcript profiling has opened up new in molecular basis of abiotic stress resistance (Wu et al. 2021). However, key limitation factors in such studies are the lack of data on metabolite flux parameters, which might be avoided by the use of powerful new instruments and protocols (Debnath et al. 2011). Similarly, high-throughput protein analyses on the stress proteome specific to one or combination of abiotic stresses are also only few in cotton (Wu et al. 2021).

Annotation and integration of data is the prerequisite to draw any meaningful and useful inferences and those efforts have helped to develop databases and statistical methods. These databases have provided essential evidences for the analysis of plant stress data such as the one developed as Plant Stress (https://plantstress.com/), although this database is not specific to the cotton abiotic stress responses. Likewise, various omics profiling database (plant-wide stress database) can be generated to compile the information on abiotic stress-dependent mechanisms and eventually this data can be used for generating new phenotypes, which can ultimately be integrated with the cotton breeding programs.

Genetic engineering of novel genes and their regulatory elements offers novel avenues in understanding various mechanism governing molecular pathways in response to one or more of the abiotic stresses and how the tolerance to abiotic stress develops in cotton. However, integrating such information in routine breeding program has not yet been fully realized its potential mainly due to the expertise and investment involved in cotton transformation and other policy issues.

4.6.2 Genome Sequencing and Resequencing

Advances in next generation sequencing technologies have also attracted attention from both cotton academia and industry and D-genome diploid cotton (*G. raimondii*) was the first effort in cotton genome sequencing in 2012 (Table 4.1). As on date (Dec., 20,211), more than one dozen cultivated and uncultivated species (among the 52 cotton species) have been fully sequenced, and hundreds of cotton accessions have been resequenced (Fang et al. 2017; Ma et al. 2018).

In order to effectively integrated the genomic information into the genetic improvement of cotton, it is essential to compare the genome structure and gene expression pattern among the sequenced genomes as well as the copy number variation of many genes (orthologs and paralogs) during cotton evolution and domestication. Extending such studies into wild cotton species and other cotton gene pools (see above) will help us in better understanding of the critical agriculturally important traits, such as yield, quality and quantity under drought, salinity and UV-light stresses.

So far, cotton genome sequence analyses have reported genes and biological processes associated with fiber qualities and very few reports have provided information on abiotic stress responsive genes and their pathways. For example, resequencing of 737 cultivars identified genes that are associated to cotton fiber growth and development as well as lint yield. It has been unarguably established that lint yield has been repeatedly selected than other traits during the long history of cotton evolution and domestication (Fang et al. 2017; Ma et al. 2018). In fact, the number of fiber-associated genes were doubled owing to whole chromosome duplication and polyploidization in cotton and elevated expression levels of fiber-related genes were detected in cultivated cotton while comparing its wild counterparts. Genome sequencing in cotton also provides an array of disease-resistant genes in cotton that have evolved over time as a result of selection and domestication (Chen et al. 2020). Additionally, sequencing analysis further showed the epigenomic regulation such as

Table 4.1 Genomic features of wild ancestors and cultivated cotton available in public domain

Species/genomic features	Genome type	Size of the genome (Gb)	Highest number of genes reported	% of repeat sequences	Reference(s)
G. raimondii	DD	0.740	40,976	60.0	Paterson et al. (2012); Wang et al. (2012)
G. arboreum	AA	1.719	41,330	68.5	Li et al. (2014)
G. barbadense	AADD	2.196–2.570	80,876	–	Liu et al. (2015)
G. hirsutum	AADD	2.305	76,943	67.2	Chen et al. (2020); Fang et al. (2017); Ma et al. (2018)

DNA methylation and small regulatory RNAs, particularly for cotton fiber development under environmental stresses (Song et al. 2017; Chen et al. 2020). Information on epigenetic inheritance would be a novel way to enhance cotton's fiber yield and quality under changing climatic conditions, however, a detailed comprehensive study towards this direction has yet to be demonstrated in cotton.

4.6.3 Panomics

Accumulating knowledge on next generation sequencing implies that a single reference genome is inadequate to unravel the full potentials of genetic diversity exist in species and hence nowadays it is proposed to focus on pan genomics. Pangenome denotes to the full complement of genes in a biological group, which can be divided into a set of central genes that are shared by all the individuals of the given species and a set of unique genes that are partially shared or distinct to a single individual of the investigated species (Tettelin et al. 2005). Pan-genome analysis (or simply panomics) thus provide a comprehensive evaluation procedure to sampling a species genetic diversity by examining the entire genome repertoire that exists in the constituent accessions of the given species.

Reference genomes available for cultivated common crops has facilitated genome-wide identification of single nucleotide polymorphisms (SNPs) followed by marker–trait association efforts to associate genetic variation with phenotypic variation. In contrast, unravelling genetic variation using resequencing approaches based on a single reference genome has been inadequate as this effort has poor capacity to identify structural variations (SVs).

Increasing evidences has repeatedly shown that SVs comprising copy number variants (CNVs) and presence/absence variants (PAVs), are widespread in agricultural plants and play essential roles in the genetic determination of desirable traits. Although advances in panomics have revealed novel information on crop diversity, the potential applications of crop panomics in crop improvement are yet to be fully exploited (Tao et al. 2019) and the same trend is prevailing in evolution of resilient cotton for abiotic stresses using panomics.

However, few studies have shown the power of panomics to unravel molecular aspects of evolution in cotton. For example, 243 *G. arboreum* and *G. herbaceum* accessions were re-sequenced to generate a genetic map of genomic variants and found that they are correspondingly diverged from *G. raimondii* (Du et al. 2018). Genome-wide association study (GWAS) located 98 significant peak associations for 11 key agronomic traits in *G. arboreum.* Such effort has also offered evidence that generate deeper knowledge on evolution of the A genome of cotton. Besides, Du et al. (2018) also have reported that nonsynonymous alteration (cysteine-to-arginine substitution) of *GaKASIII* appears to confer significant fatty acid composition (C16:0 and C16:1) modification in cotton seeds as well as resistance to Fusarium wilt disease, which might be due to the activation of *GaGSTF9* expression.

4.6.4 High-Density Genetic Maps and High-Resolution Trait Dissection

Immediate applications of genome research are to increase plant breeding efficiency by providing powerful tools to dissect and introgress complex traits genetically. Marker-assisted selection and genome selection using genetic markers closely linked to target genes or quantitative trait loci (QTLs) are few examples (Boopathi 2020). The primary prerequisite for QTL identification and consequent MAS is availability of informative molecular markers and high-throughput genotyping platforms as they play an important role in such efforts.

In cotton, simple sequence repeat (SSR) markers are the most commonly used molecular marker. So far, in cotton database, Cotton DB (http://cottondb.org/) 19,010 SSR markers have been recorded; besides 100,290 microsatellites have been mined in the genome of *G. hirsutum*, from which 77,996 SSR markers have been developed and confirmed (Wang et al. 2015). In order to saturate cotton genetic maps (referred as high-density genetic maps) and to significantly enhance gene/QTL mapping for genomic studies and MAS, high throughput molecular markers are required. SNPs are one these marker classes because they are the most abundant (theoretically unlimited) DNA sequence variation present in plant genomes, and they are evenly distributed throughout the genome, bi-allelic, codominant and amenable for automation. Several studies have been reported on genome-wide SNP marker development and their application in cotton. In *G. hirsutum*, a total of 132,262 intraspecific SNPs has been developed, out of which 223,138and 470,631 interspecific SNPs have been documented between *G. hirsutum* and *G. barbadense* or *G. longicalyx*, respectively (Liu et al. 2018). According to Cai et al. (2017) catalogued 82,259 SNP markers were used from the re-sequencing data of 100 cotton cultivars and were utilized to develop the CottonSNP80K array on the Illumina Infinium high-throughput platform and demonstrated its utilization in genotyping detection in diverse cotton accessions. These findings imply that high-density linkage maps with automated SNPs play an important role in cotton genetics and breeding studies.

Despite of the development of high-density genetic maps in cotton, the majority of these studies have been focused on to identifying QTLs for yield, fiber quality and their component traits under normal production conditions while only a few studies have been done to identify QTLs linked to abiotic stress tolerance.

Jiang et al. (2000) mapped 40 QTLs associated to leaf morphology, and the major QTL cluster was found on chromosome c15, corresponding to the Okra-leaf, a trait that was previously shown to be linked with drought tolerance. It was also found that locus on chromosome c6 influence trichrome density on leaves, thereby affecting the transpiration rate under drought stress.

Saranga et al. (2001, 2004) identified 33 QTLs associated with drought tolerance traits such as osmotic potential, carbon isotope ratio (^{13}C), dry matter, canopy temperature, and chlorophyll a and b content, fiber length, seed cotton yield, harvest index, length uniformity, boll weight and number, fineness (micronaire), strength, elongation, and color components (reflectance and yellowness) under drought stress.

However, it was discovered that only decreased osmotic adjustment contributed to increased seed cotton output under drought stress, showing the presence of a phenotypic link between physiological and productive traits. Together with the previous study and the findings of Levi et al. (2009), showed improvement of polygenic traits via marker-assisted backcrossing is a big challenge in case of near-isogenic lines (NILs) population grown under well-watered and water-limited conditions in a multi-environment field condition.

According to Oluoch et al. (2016) 11 QTLs were found associated with salt tolerance under laboratory conditions. Zheng et al. (2016) utilized the same population to identify QTLs for drought tolerance in the field condition and reported that 67 and 35 QTLs under water-limited and well-watered conditions, respectively. Similarly, Diouf et al. (2017) revealed 66 QTLs located in different levels of salt treatments (0-, 110-, and 150-mM) out of which 14 of them were found to be consistent, even though they are accounting for 2.72% to 9.97% of the phenotypic variation.

Using SSR markers, Jia et al. (2014) demonstrated that three SSR markers are highly associated with regards to salt tolerance while 15 markers for drought tolerance and both the markers are not overlapping each other. Additionally, according to Du et al. (2016) 95 loci were found to be significantly associated with salt tolerance traits, although there was no consistent and significant QTLs with higher phenotypic variation, that is required for MAS.

Meta-analysis of 661 abiotic and biotic stress resistance QTLs using the Bio Mercator software identified chromosomal regions containing QTL clusters for different resistance traits in cotton: 98 QTLs for drought tolerance in the greenhouse and 150 QTLs in field conditions, 80 QTLs for salt tolerance in the greenhouse conditions, 201 QTLs for Verticillium wilt, 47 QTLs for Fusarium wilt and 85 QTLs for root-knot nematodes and reniform nematodes resistance (Abdelraheem et al. 2017). Most importantly, two QTL hotspots for chlorophyll content assessed under drought and salt tolerance using a SPAD meter were identified on chromosome c24. Other traits such as morphological traits (plant height and fresh and dry shoot and root weighs), physiological traits (osmotic potential, chlorophyll content, carbon isotope ratio, stomatal conductance, photosynthetic rate, transpiration, canopy temperature, and leaf area index), fiber quality trait (fiber length, uniformity, strength, elongation and micronaire), agronomical traits (seed cotton yield, boll weight, lint yield, and lint percentage) are used in QTL mapping for abiotic stress.. The results demonstrated that desirable abiotic resistant QTLs are not evenly distributed across the cotton genome; with certain chromosomes having much more QTLs, QTL clusters, or hotspots than others.

Despite of the above progresses, till date none of the QTL governing abiotic stress tolerance has been used in cotton breeding program through MAS. Both intraspecific and interspecific mapping population employing varied and multiple parents should be established in order to develop robust cotton cultivar using MAS (Ravelombola et al. 2021). These populations are required for doing repeated phenotypic analysis for abiotic stress tolerance and thus deriving high-resolution QTL mapping. In addition, to increase the consistency and scalability of phenotyping of cotton mapping population for drought and salt stress resistance, rapid, reliable, and high

throughput screening methodologies suitable to large populations must be developed (Abdelraheem et al. 2019).

Cutting-edge technologies for genome sequencing and phenotyping of crops under abiotic stress conditions in combination with machine learning, have recently increase the possibility of identifying the genetic basis of traits with unprecedented precision and providing useful information for decision-making towards achieving breeding targets. Esposito et al. (2020) summarized current advances in next-generation sequencing as well as the use of phenotyping technologies in genomics-assisted breeding in order to identify targeted genes governing abiotic stress and also to control the exploitation of the natural variation.

Particularly, the key in high-resolution trait mapping is the use of high-throughput strategies for phenotyping of large numbers of plant materials for abiotic stress resistance. According to Kashiwagi et al. (2015), root traits, such as root length density played a major role in mitigating the effect of drought and improving yield by studying the root system architecture and its plasticity in chickpea germplasm lines. The optimal selection method for yield in terminal drought conditions in chickpea was found to show profuse root length density, root dry weight, and a high root-to-shoot ratio (Bharadwaj et al., 2021). However, phenotyping for the cotton root traits, especially under heterogenous drought stressed soil conditions, requires heavy investments and painstaking time and effort. This implies that a lot has to be done cotton root phenomics before employing genomics assisted breeding in cotton for abiotic stress resistance improvement.

4.6.5 Comprehensive Transcriptomic and Proteomic Resources

Elaborate molecular responses are involved in the plant as a rejoinder to abiotic stresses with respect to timings and quantum of responses. In response to abiotic stresses, post-transcriptional mechanisms such as alternative splicing, RNA processing and RNA silencing influence the actual transcriptome (Balfagón et al. 2020). Besides, protein phosphorylation and other post-translational modifications such as ubiquitination and sumoylation regulate the gene expression in response to stresses. There is also cross-connections among these mechanisms and altogether it clearly indicates that response to environmental changes has superimposed complexity at multi levels.

Until now, such complex mechanisms were characterized primarily as regulatory elements of the stress–response pathways and network of post-transcriptional and post-translational alteration of downstream stress-related gene expression at temporal and spatial patterns (Wu et al. 2021). Though few transcriptomic studies have attempted in examining cotton leaf curl disease resistance in a naturally immune cotton species, *G. arboretum* (Naqvi et al. 2017; Naqvi et al. 2017), it has been clearly demonstrated for abiotic stress (such as drought (Ranjan and Sawant 2015;

Tahmasebi et al. 2019; Hasan et al. 2019) and salt (Wei et al. 2017) tolerance and ion balance (Guo et al. 2019)) in cotton and collectively inferred that multiple resistance mechanisms were involved against these stresses.

For example, Peng et al. (2018) reported that combination of proteomic and transcriptomic profiles leads to the discovery of multiple levels of genetic regulation for salt tolerance in cotton and projected a collaboration network containing 158 genes/proteins. They also discovered that ATP synthase (CotAD 74,681) and cytochrome oxidase (CotAD 46,197) in mitochondria formed two major clusters in the network. However, further efforts through genetic engineering may provide deeper insights on these complex regulation of abiotic stress responses to develop stress-resilient cotton cultivars.

4.6.6 Metabolomics

Metabolomics is a post-genomics tool to disclose physiological and biochemical rejoinders of the investigated plants under abiotic stresses (Godoy et al. 2021). Metabolites are identified mainly through mass spectrometry associated with chromatographic instrumentation and electrophoretic techniques. However, the rate limiting procedure in metabolite analysis is appropriate selection ionization method and analyzer type used in mass spectrometer and it desperately require different configuration of mass spectrometric instrumentation for useful collection of metabolomic data (Rodziewicz et al. 2014).

Non-targeted metabolomics has been used to decipher the host biochemical mechanism of quantitative resistance in crop plants to a variety of stresses, and these studies have aided in the development of meaningful findings about the defense mechanism of commercially significant crops. Though no specific abiotic stress responsive metabolomics in cotton has been reported, but for biotic stress responsive metabolites were recorded.

Different amounts of glycosides were found in different *Gossypium* sp., through metabolic profiling. For example, Rhamno glucosides are more abundantly found in *G. hirsutum* but are present in traces amount in *G. barbadense*, while kaempferol-3-glucoside and quercetin-7-glycosides are common in *G. barbadense* but not much known in *G. hirsutum* (Bolton 2009). Quercitin, gossypol, bhenic acid scopoletin, kaempferol, cinnamic acid,, quercetin-3-rhamnoglucoside, stigmasterol catechin, gallocatechin, and epicatechin have reported in several occasions in *G. hirsutum* (Nix et al. 2017). In young cotton leaves, higher concentrations of catechin, gallocatechin and iso quercitrin, has found to associated with limited mycelia growth.

In response to leaf spot disease in cotton, a detailed report on non-targeted metabolomics employing ultra-high-performance liquid chromatography–mass spectrometry found abundant levels of important metabolites such as flavonoids, phenylpropanoids, terpenoids, fatty acids, and carbohydrates (Khizar et al. 2020). Among 241 resistance related metabolites, 223 were resistance-related induced metabolites and 18 were identified as resistance-related constitutive metabolites. It

has also noted that these metabolites were the precursors (such as phenylpropanoides: stilbenes and furanocoumarin, alkaloids: indolizine and acetylcorynoline, flavonoids: phlorizin and kaempferol and terpenoids: azelaic acid and oleanolic acid) for a number of secondary metabolic pathways such. Thus, it is clearly demonstrated that secondary metabolism, primary metabolism and energy metabolism were all found to be more active in resistant cultivar when compared to sensitive cultivar. However, further studies are required to reveal the pathways engaged in defense mechanism in cotton and their utility in cotton breeding program.

4.6.7 Transgenic Approaches

Transgenic approaches offer an appropriate and alternative to conventional breeding strategy to achieve plant genetic improvements in a more targeted means. Genetic engineering proved notable developments in several occasions during the last few decades that transfer or modifications genes can have desired characteristics in the transgenic plants. Transgenic approaches aid in the precise identification of the candidate genes, miRNAs, and transcription factors (TFs) involved in specific abiotic stress responses, enlightening about the molecular and physiological aspects of plants in changing climatic conditions. Molecular breeding via transgenics has proven to be a promising tool for abiotic stress improvement in several commercial crops and the accuracy and precision of this approach offer promises for the future plant breeding programs (Anwar and Kim 2020). The below lines exemplify some of the key success in cotton towards abiotic stress resistance improvement.

Transcription factors (TFs) are the key genes that are involved in plant response to abiotic stresses. Overexpression of *GhABF2*, a *bZIP* transcription factor gene, in cotton, showed drought and salt tolerance through ABA-related gene regulation. Cotton plants overexpressing *GhABF2* produced higher yields than non-transgenic control plants (Liang et al. 2016). Similar kind of experiments have been outlined in Abdelraheem et al. (2019 and references therein) where *AtRAV1/2* (a basic 3-DNA-binding domain) and *AtABI5*(a basic leucine zipper) transcription factor gene, were overexpressed, resulting in an improved drought tolerant phenotype in cotton. Overexpression of the rice *SNAC1* (member of the NAC family of transcription factors gene) was in cotton plants showed more drought and salt tolerant as they produced more robust rooting system having low transpiration rate as compared to comparison non-transgenic cotton plants. Likewise, overexpression of the *Arabidopsis ENHANCED DROUGHT TOLERANCE1/HOMEODOMAIN GLABROUS11 (AtEDT1/HDG11)* gene in cotton showed increased drought and salt tolerance.

Overexpression of *TsVP*, a *H + -PPase* gene from *Thellungiella halophile*, in cotton shows improved drought tolerance and increased growth under salt stress conditions as compared to non-transgenic cotton because these genes lead to increase in photosynthetic rates (Lv et al. 2009). Similarly, Shen et al. (2015) reported that by using*AVP1* and *AtNHX1* genes in cotton an improved salt and drought tolerance

transgenic cotton were produced. Under low-irrigation and dryland circumstances, transgenic cotton plants produced 24% and 35% more fiber than non-transgenic cotton plants, respectively.

Overexpression of the *isopentenyl transferase* gene (*IPT*), which is involved in cytokinin biosynthesis, in cotton revealed that the yield of transgenic cotton was strongly influenced by the timing of the drought stress as IPT-transgenic cotton outperforms in the non-transgenic cotton during drought condition and also before flowering stage (Zhu et al. 2018). Also, overexpression of IPT leads to salt tolerance and increase in biomass in non-transgenic cotton plants (Liu et al. 2012).According to Mishra et al. (2017) overexpression of the rice *OsSIZ*, *SUMO E3 ligase* gene in cotton result in tolerance towards drought stress, thereby, fiber yield is increased in a dryland agricultural system.

Similar to overexpression of the selected genes, suppression of certain genes also led to abiotic stress tolerance in cotton. For example, Chen et al. (2015) pointed out that downregulation of *GbMYb5* is due to the deactivation of POD, CAT, and SOD enzymes, as a result, oxidative stress in drought condition is eventually increased.

Thus, transgenic technology looks to be a powerful and impactful strategy for improving crop tolerance to abiotic challenges such as salinity and water stress. However, most of these studies were conducted either in laboratory or under greenhouse conditions and performance of these abiotic stress resistance transgenic cotton under real field conditions with varying degree of time, intensity and duration of stresses yet to be demonstrated.

4.7 Progresses in Translational Genomics

Developments in sequencing and genotyping technologies have resulted in the abundant generation of genomic resources in cotton. Studies in 'Translational genomics' should be carried out in order to transfer such vast genomic knowledge to useful products in the farmer's field. The transferred genomic information might serve as valuable hints for breeding and functional biology, that help to attain objectives such as improved yield, pest and disease resistance besides providing sustainable yield under abiotic stresses.

Alongside with reference genomes, genetic and genomic resources in diverse cotton germplasm can be enriched by genome-wide analyses using resequencing and genotyping techniques, researchers were able to uncover previously unknown associations between genomic variations and diverse phenotypes in cotton. This enhanced genetic knowledge must be transmitted into the crop breeding field in order to allow for precise breeding and quick breeding methods. It is suggested that genetic studies should be preserved and compiled in the form of a database with showing phenotype-based categorization and its respective genomic markers along with the positions. If their genetic data are curated as the database following the phenotype-driven database format (for example, genomic hot spots for abiotic stress resistance), the database would be additional model database for the translational

genomics. Besides, this information can also be utilized for comparative genomic study in order to reveal highly conserved gene families across plant species.

4.7.1 Rapid Generation Turnover Technologies and Speed Breeding

Rapid generation technology (RGT) can minimize the time it takes to develop new varieties by shortening the generation cycles. Usually, development of homozygous lines from segregating populations derived from hybridization takes seven to nine years, if only one generation is produced per year. Plant breeders have used a variety of strategies to speed up generation turnover in order to overcome the considerable time it takes to produce homozygous lines. For example, effective breeding programs in lentils resulted in the production of the crops two to three generation annually: one crop during regular season in the field and the remaining two generations in a greenhouse/growth chamber/contra-season nursery in the off-season. On the other hand, RGT allows faster generation turnover by annually producing up to 6.8 generations for faba bean and 8 generations for lentil (Mobini et al. 2015).

Accelerated flowering and regeneration advancement has also been demonstrated in different genotypes of pea, velvet bean, tomato. For example, Saxena et al. (2019) provided a single seed descent approach and a speed breeding strategy that efficiently utilizes immature seed germination for rapid generation advancement. In order to hasten desirable line development at the same time safeguarding genetic variability, RGT enables four generations in a year and can facilitate faster field evaluation of resulting homozygous lines. Thus, RGT not only help to save resources but it can also be employed to release elite early maturing cultivars within a short period of time.

However, employing speed breeding strategy in cotton is yet to be demonstrated. Even two to three crops in a year, would greatly reduce effort and time in evolving cotton cultivars with improved abiotic stress resistance.

4.7.2 Genomic Selection

Genotyping assays with various marker density are essential to establish low-cost platform when an application-oriented breeding research is planned. For example, low-density assay (10–100 polymorphic SNPs) can be used to test the purity of founder parents and their offspring in breeding programs. However, for genomic selection, it may need a mid-density assay (2000–5000 SNPs) and for performing genetic diversity, genome-wide association and genetic mapping studies, a high-density assay with >10 K SNPs is required (Zhong et al. 2021).

During the genomic selection, the use of high-density SNP assay resulted in a significant diversity loss in cultivated gene pool and favored selection for different subspecies besides discovery of positive selection in genomic regions during breeding. High-density mapping can be achieved using whole genome resequencing and skim sequencing, while GBS, restriction site associated sequencing, specific length amplified fragment sequencing leads to mid-density genotyping technology (Bharadwaj et al. 2021). In the coming years, cost-effective low-density and mid-density genotyping assays for various genetic and breeding applications in cotton will be developed and deployed.

Among the different pooled sequencing-based approaches (like QTL-Seq, MutMap, Seq-BSA InDel-Seq and BSR-Seq), the 'QTL-Seq' method was successfully used to discover genomic regions and candidate genes in groundnut (Pandey et al. 2016). However, very few studies have employed these approaches in cotton. For example, Wang et al. (2021) identified a discrete genomic region on chromosome D08 with a large physical interval (~53 Mb length) and low recombination frequency that was significantly associated with fiber strength. However, using various techniques they found that these regions were introgressed from wild diploid cotton, G. *thurberi*, accounting for nearly 77% similarity in chromosome D08. They also provided molecular evidence for fiber strength improvement caused by this introgression. However, no report on impact of introgression of genomic segments from either cultivated or from alien has been found.

Out of the three genomics-assisted breeding (GAB) techniques (namely marker-assisted selection or marker-assisted backcrossing, genome selection and marker-assisted recurrent selection), the Marker-assisted selection/Marker-assisted backcrossing has proved to be the most prevailing approaches in most of the crops (Bharadwaj et al. 2021), while in cotton these techniques are still in the optimization stage. Particularly, the effort to transfer abiotic stress resistance QTLs through MAS or MABC has not yet been tried.

Thus, in order to speed up the process to realize accelerated breeding gain in cotton resilient to abiotic stress resistance, several technologies have to be employed in cotton breeding program. Such technologies include (a) sequence-based trait mapping and breeding, (b) genome editing, (c) artificial intelligence, (d) early generation development and robust screening procedures, (e) speed breeding, (f) digitalization, (g) genomic selection, (h) low-cost genotyping assays, (i) setting up of quality checked (QC) panel, (j) faster variety replacement, (k) haplotype-based breeding and (l) operational efficiency including mechanization in boll harvesting and automation in fiber quality assessment.

4.7.3 Advances in Hybrid Breeding

Though considerable progress has been achieved in cotton hybrid production, knowledge on molecular mechanism governing hybridization producing better yield in

cotton is critical for efficient breeding programs. In upland cotton through genome-wide comparative transcriptomics Shahzad et al. (2020) identified nine key genes and pathways associated with biological process of yield heterosis.

Third-generation hybrid rice breeding system has been recently reported by Wang and Deng (2018) that employs transgenics to evolve stable recessive nuclear male sterile line. On the other hand, the products of such effort have been considered as non-transgenic due to the mechanism involved in this procedure. In order to generate maintainer line, mutant plant showing male sterility is incorporated with fertility restoration gene along with a seed color gene and a pollen lethality gene (all three genes are intertwined). As a result, the progenies generated from maintainer line would segregate into half male sterile and half colored maintainer seeds thereby segregating seeds using colour-sorting machine. Consequently, the hybridization between the male sterile line and the pollen lethality maintainer would produce 100% pure male sterile lines (Wang and Deng 2018).

It is believed that this third-generation hybrids can surpass the fundamental issues of the first- and second-generation hybrid rice systems and offer strategies to further raise the production not only in rice but also in other crops that are responsive to transgenic methods. Especially evolving such third-generation hybrids with improved abiotic stress resistance can have great impact in increasing the cotton fiber productivity under fragile environments where abiotic stresses severely hamper the fiber yield.

4.8 Prospects for Delivering Genomics to Marginal Farmers

From the above preceding paragraphs, it is obvious that combination of different genomic technologies will result in the faster delivery of climate change ready cotton cultivars. However, to realize the genomics potentials in evolving abiotic stress resilient cotton cultivar, intensive technical investments in (i) cotton germplasm characterization (to identify superior alleles/haplotypes through next generation sequencing based approaches), (ii) precise phenotyping (for effective association of abiotic stress resistance traits and genomic regions) and (iii) development of Pre-breeding cotton progenies through genomics-assisted breeding approaches, are required. It is anticipated that such efforts will result in the quick delivery of climate change ready cotton cultivars.

Though sequencing/genotyping of the entire cotton gene bank of cotton will be ideal, smaller subsets of germplasm (core or mini core collection) can be targeted which will be cost effective. Likewise, specific cotton genetic resources and/or genetic stocks such as bi-parental (recombinant inbred lines, backcross progenies, introgression lines, F_2progenies) and multi-parental (nested association mapping and multi-parent advanced generation intercross) populations segregating for abiotic stress resistance related traits can be utilized. A training population based on the

specialized set of breeding lines can also be produced for genomic selection implementation. These resources can be used for whole genome resequencing or high-density genotyping and high-throughput phenotyping of the same resources especially for abiotic stress resistance/tolerance traits. Using recent improvements in sensors and imaging-based phenotyping technology, this may be done in many situations using visible light, the near-infrared spectrum, and fluorescence imaging.

Quantitative trait loci, marker trait associations, quantitative trait nucleotides (QTN), and genomic estimated breeding value can be obtained by combining sequencing or genotyping data with phenotyping data utilizing analytical and decision support tools. Further, such effort will lead to find a catalogue of superior haplotypes and source donors for abiotic stress resistance traits. Such information can be employed in pre-breeding and genomics assisted breeding. To this end, new donor plant can introgress new alleles in to the selected/cultivated cotton gene pool from the unadopted germplasm (wild types, landraces, and ecotypes) originating from extreme environmental conditions.

As a result, in addition to produce better pre-breeding lines, the elite cotton gene pool genetic diversity can also be enlarged by accumulating superior alleles for abiotic stress resistance. Further, QTNs can also be modified using genome editing approach (i.e., promotion of alleles through genome editing). Integration of such approaches in routine breeding program will accelerate the release of climate-resilient cultivars with improved yield, increased resistance/tolerance to anticipated abiotic stresses and higher genetics gains in farmer's fields particularly in resource-constrained environments where normally cotton is grown.

References

Abdelraheem A, Liu F, Song M, Zhang JF (2017) A meta-analysis of quantitative trait loci for abiotic and biotic stress resistance in tetraploid cotton. Mol Genet Genom 292(6):1221–1235

Abdelraheem A, Esmaeili N, O'Connell M, Zhang J (2019) Progress and perspective on drought and salt stress tolerance in cotton. Ind Crops Prod 130:118–129

Allison DC, Fisher WD (1964) A dominant gene for male-sterility in upland cotton 1. Crop Sci 4(5):548–549

Anwar A, Kim JK (2020) Transgenic breeding approaches for improving abiotic stress tolerance: recent progress and future perspectives. Intl J Mol Sci 21(8):2695

Baruah PM, Krishnatreya DB, Bordoloi KS, Gill SS, Agarwala N (2021) Genome wide identification and characterization of abiotic stress responsive lncRNAs in Capsicum annuum. Plant Physiol Biochem162:221–236

Basal H, Hemphill JK, Smith CW (2006) Shoot and root characteristics of converted race stocks accessions of upland cotton (*Gossypium hirsutum* L.) grown under salt stress conditions. Am J Plant Pathol 1(1):99–106

Beasley JO (1940) The origin of American tetraploid *Gossypium* species. Am Nat 74(752):285–286

Bharadwaj C, Tripathi S, Soren KR, Thudi M, Singh RK et al. (2021) Introgression of "QTL-hotspot" region enhances drought tolerance and grain yield in three elite chickpea cultivars. Plant Genome: e20076

Bolton MD (2009) Primary metabolism and plant defense—fuel for the fire. Mol Plant-Microbe Interact 22(5):487–497

Boopathi NM (2020) Marker-assisted selection (MAS). In: Boopathi NM (ed) Genetic mapping and marker assisted selection. Springer, Singapore, pp 343–388
Chastain DR, Snider JL, Choinski JS, Collins GD, Perry CD et al (2016) Leaf ontogeny strongly influences photosynthetic tolerance to drought and high temperature in *Gossypium hirsutum*. J Plant Physiol 199:18–28
Chen T, Li W, Hu X, Guo J, Liu A, Zhang B (2015) A cotton MYB transcription factor, GbMYB5, is positively involved in plant adaptive response to drought stress. Plant Cell Physiol 56(5):917–929
Chen ZJ, Sreedasyam A, Ando A, Song Q, De Santiago LM, Hulse-Kemp AM et al (2020) Genomic diversifications of five Gossypium allopolyploid species and their impact on cotton improvement. Nat Genet 52(5):525–533
Debnath M, Pandey M, Bisen PS (2011) An omics approach to understand the plant abiotic stress. OMICS 15(11):739–762
Dewi ES (2011) Root morphology of drought resistance in cotton (*Gossypium hirsutum* L.). Doctoral dissertation, Texas A & M University
Du X, Huang G, He S, Yang Z, Sun G, Ma X et al (2018) Resequencing of 243 diploid cotton accessions based on an updated A genome identifies the genetic basis of key agronomic traits. Nat Genet 50(6):796–802
Du L, Cai C, Wu S, Zhang F, Hou S, Guo W (2016) Evaluation and exploration of favorable QTL alleles for salt stress related traits in cotton cultivars (*G. hirsutum* L.). PLoS One 11(3):e0151076
Edmeades GO, Cooper M, Lafitte R, Zinselmeier C, Ribaut JM, Habben JE, Loffler C, Banziger M (2001) Abiotic stresses and staple crops. In: Nosberger J, Geiger HH, Struik PC (eds) Crop science, progress and prospects. Proceedings of the third international crops science congress, 17–21 Aug 2000. CABI, Wallingford, UK, pp 137–154
Fang L, Wang Q, Hu Y, Jia Y, Chen J et al (2017) Genomic analyses in cotton identify signatures of selection and loci associated with fiber quality and yield traits. Nat Genet 49(7):1089
Fryxell PA (1992)A revised taxonomic interpretation of *Gossypium* L (Malvaceae). Rheedea 2:108–116
Godoy F, Olivos-Hernández K, Stange C, Handford M (2021) Abiotic stress in crop species: Improving tolerance by applying plant metabolites. Plants10(2):186
Gotmare V, Singh P (2004) Use of wild species for cotton improvement in India. ICAC Rec 22:12–14
Guo H, Li S, Min W, Ye J, Hou Z (2019) Ionomic and transcriptomic analyses of two cotton cultivars (*Gossypium hirsutum* L.) provide insights into the ion balance mechanism of cotton under salt stress. PLoSOne 14(12): e0226776
Gutiérrez RA, Shasha DE, Coruzzi GM (2005) Systems biology for the virtual plant. Plant Physiol 138(2):550–554
Hamdy EM, Barghouti S, Gillham F, AI-Saffy T (1994) Cotton production prospects for the decade to 2005: a global overview. World Bank Technical Paper No.231
Harlan JR, de Wet JM (1971) Toward a rational classification of cultivated plants. Taxon 20(4):509–517
Harlan JR (1992) Classification of cultivated plants. In: Harlan (ed) Crops & Man, pp 101–114
Hasan MM, Ma F, Islam F, Sajid M, Prodhan ZH et al. (2019) Comparative transcriptomic analysis of biological process and key pathway in three cotton (*Gossypium* spp.) species under drought stress. Intl J Mol Sci 20(9):2076
Hedhly A, Hormaza JI, Herrero M (2009) Global warming and sexual plant reproduction. Trends Plant Sci 14(1):30–36
Jauhar PP (2006) Modern biotechnology as an integral supplement to conventional plant breeding: the prospects and challenges. Crop Sci 46(5):1841–1859
Jia YH, Sun JL, Wang XW, Zhou ZL, Pan ZE, He SP et al. (2014) Molecular diversity and association analysis of drought and salt tolerance in *Gossypium hirsutum* L. germplasm. J Integr Agri 13(9):1845–1853
Jiang C, Wright RJ, Woo SS, DelMonte TA, Paterson AH (2000) QTL analysis of leaf morphology in tetraploid *Gossypium* (cotton). Theor Appl Genet 100(3–4):409–418

Justus N, Leinwebr CL (1960) A heritable partially male-sterile character in cotton. J Hered 51:191–192

Kashiwagi J, Krishnamurthy L, Purushothaman R, Upadhyaya HD, Gaur PM et al (2015) Scope for improvement of yield under drought through the root traits in chickpea (*Cicer arietinum* L.). Field Crops Res 174:47–54

Kerr T (1960) The trispecies hybrid ancestry of high strength cottons. In: Proceedings of cotton improvement conference 21, p 82

Khizar M, Shi J, Saleem S, Liaquat F, Ashraf M, Latif S et al (2020) Resistance associated metabolite profiling of Aspergillus leaf spot in cotton through non-targeted metabolomics. PLoSOne 15(2):e0228675

Kohel RJ (1974) Influence of certain morphological characters on yield. Cotton Grow Rev 51:281–292

Kulkarni VN, Khadi BM, Maralappanavar MS, Deshapande LA, Narayanan SS (2009) The worldwide gene pools of *Gossypium arboreum* L. and *G. herbaceum* L., and their improvement. In: Paterson AH (ed) Genetics and genomics of cotton. Springer, New York, NY, pp 69–97

Larcher W (2003) Physiological plant ecology: ecophysiology and stress physiology of functional groups. Springer Science & Business Media

Levi A, Paterson AH, Barak V, Yakir D, Wang B, Chee PW, Saranga Y (2009) Field evaluation of cotton near-isogenic lines introgressed with QTLs for productivity and drought related traits. Mol Breed 23(2):179–195

Li F, Fan G, Wang K, Sun F, Yuan Y, Song G et al (2014) Genome sequence of the cultivated cotton *Gossypium arboreum*. Nat Genet 46(6):567–572

Liang C, Meng Z, Meng Z, Malik W, Yan R, Lwin KM et al (2016) GhABF2, a bZIP transcription factor, confers drought and salinity tolerance in cotton (*Gossypium hirsutum* L.). Sci Rep 6(1):1–14

Liu YD, Yin ZJ, Yu JW, Li J, Wei HL, Han XL et al (2012) Improved salt tolerance and delayed leaf senescence in transgenic cotton expressing the *Agrobacterium* IPT gene. Biol Planta 56(2):237–246

Liu X, Zhao B, Zheng HJ, Hu Y, Lu G, Yang CQ et al (2015) *Gossypium barbadense* genome sequence provides insight into the evolution of extra-long staple fiber and specialized metabolites. Sci Rep 5(1):1–14

Liu R, Gong J, Xiao X, Zhang Z, Li J, Liu A et al (2018) GWAS analysis and QTL identification of fiber quality traits and yield components in upland cotton using enriched high-density SNP markers. Front Plant Sci 9:1067

Loka DA, Oosterhuis DM, Ritchie GL (2011) Water-deficit stress in cotton. Stress Physiol Cotton 7:37–72

Luo HH, Zhang YL, Zhang WF (2016) Effects of water stress and rewatering on photosynthesis, root activity, and yield of cotton with drip irrigation under mulch. Photosynthetica 54(1):65–73

Lv SL, Lian LJ, Tao PL, Li ZX, Zhang KW, Zhang JR (2009) Overexpression of *Thellungiella halophila* H+-PPase (TsVP) in cotton enhances drought stress resistance of plants. Planta 229(4):899–910

Ma Z, He S, Wang X, Sun J, Zhang Y, Zhang G et al (2018) Resequencing a core collection of upland cotton identifies genomic variation and loci influencing fiber quality and yield. Nat Genet 50(6):803–813

McMichael BL, Quisenberry JE (1991) Genetic variation for root-shoot relationships among cotton germplasm. Environ Exp Bot 31(4):461–470

Meredith WR Jr (1990) Yield and fiber-quality potential for second-generation cotton hybrids. Crop Sci 30(5):1045–1048

Meredith Jr WR, Bridge RR(1971) Breakup of linkage blocks in cotton, *Gossypium hirsutum* L. Crop Sci 11(5):695–698

Mishra N, Sun L, Zhu X, Smith J, Srivastava A et al (2017) Overexpression of the rice SUMO E3 ligase gene OsSIZ1 in cotton enhances drought and heat tolerance, and substantially improves

fiber yields in the field under reduced irrigation and rainfed conditions. Plant Cell Physiol 58(4):735–746
Mobini SH, Lulsdorf M, Warkentin TD, Vandenberg A (2015) Plant growth regulators improve in vitro flowering and rapid generation advancement in lentil and faba bean. In Vitro Cell Dev BiolPlant 51(1):71–79
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681
Naqvi RZ, Zaidi SSEA, Akhtar KP, Strickler S, Woldemariam M et al (2017) Transcriptomics reveals multiple resistance mechanisms against cotton leaf curl disease in a naturally immune cotton species, *Gossypium arboreum*. Sci Rep 7(1):1–15
Nix A, Paull C, Colgrave M (2017) Flavonoid profile of the cotton plant, *Gossypiumhirsutum*: a review. Plants 6(4):43
OECD/FAO (2018) OECD-FAO agricultural outlook. OECD Agriculture statistics (database). https://doi.org/10.1787/agr-outl-data-en
Pace PF, Cralle HT, El-Halawany SH, Cothren JT, Senseman SA (1999) Drought-induced changes in shoot and root growth of young cotton plants. J Cotton Sci 3(4):183–187
Pandey MK, Roorkiwal M, Singh VK, Ramalingam A, Kudapa H et al (2016) Emerging genomic tools for legume breeding: current status and future prospects. Front Plant Sci 7:455
Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J et al (2012) Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. Nature 492(7429):423–427
Peng Z, He S, Gong W, Xu F, Pan Z et al (2018) Integration of proteomic and transcriptomic profiles reveals multiple levels of genetic regulation of salt tolerance in cotton. BMC Plant Biol 18(1):1–19
Penna JCV, Verhalen LM, Kirkham MB, McNew RW (1998) Screening cotton genotypes for seedling drought tolerance. Genet Mol Biol 21:545–549
Pettigrew WT (2004) Physiological consequences of moisture deficit stress in cotton. Crop Sci 44(4):1265–1272
Ramadan M, Alariqi M, Ma Y, Li Y, Liu Z et al (2021) Efficient CRISPR/Cas9 mediated pooled-sgRNAs assembly accelerates targeting multiple genes related to male sterility in cotton. Plant Methods 17(1):1–13
Ranjan A, Sawant S (2015) Genome-wide transcriptomic comparison of cotton (*Gossypium herbaceum*) leaf and root under drought stress. Biotechnology 5(4):585–596
Richmond TR, Kohel RJ (1961) Analysis of a completely male-sterile character in American upland cotton 1. Crop Sci 1(6):397–401
Rodziewicz P, Swarcewicz B, Chmielewska K, Wojakowska A, Stobiecki M (2014) Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol Planta 36(1):1–19
Saranga Y, Menz M, Jiang CX, Wright RJ, Yakir D, Paterson AH (2001) Genomic dissection of genotype × environment interactions conferring adaptation of cotton to arid conditions. Genome Res 11(12):1988–1995
Saranga YE, Jiang CX, Wright RJ, Yakir D, Paterson AH (2004) Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. Plant Cell Environ 27(3):263–277
Saranga Y, Paterson AH, Levi A (2009) Bridging classical and molecular genetics of abiotic stress resistance in cotton. In: Paterson AH (ed) Genetics and genomics of cotton. Springer, New York, NY, pp 337–352
Saxena KB, Saxena RK, Hickey LT, Varshney RK (2019) Can a speed breeding approach accelerate genetic gain in pigeonpea? Euphytica 215(12):1–7
Shahzad K, Zhang X, Guo L, Qi T, Bao L et al (2020) Comparative transcriptome analysis between inbred and hybrids reveals molecular insights into yield heterosis of upland cotton. BMC Plant Biol 20:1–18
Shen G, Wei J, Qiu X, Hu R, Kuppu et al (2015) Co-overexpression of AVP1 and AtNHX1 in cotton further improves drought and salt tolerance in transgenic cotton plants. Plant Mol Biol Rep 33(2):167–177

Song Q, Zhang T, Stelly DM, Chen ZJ (2017) Epigenomic and functional analyses reveal roles of epialleles in the loss of photoperiod sensitivity during domestication of allotetraploid cottons. Genome Biol 18(1):1–14

Stewart JMCD (1995) Potential for crop improvement with exotic germplasm and genetic engineering. In: Proceeding of the world cotton research conference-I, Brishbane, Melbourne, Australia, February 14–17, pp 313–327

Stiller WN, Read JJ, Constable GA, Reid PE (2005) Selection for water use efficiency traits in a cotton breeding program: cultivar differences. Crop Sci 45(3):1107–1113

Tahmasebi A, Ashrafi-Dehkordi E, Shahriari AG, Mazloomi SM, Ebrahimie E (2019) Integrative meta-analysis of transcriptomic responses to abiotic stress in cotton. Progress Bio Phys Mol Biol 146:112–122

Tao Y, Zhao X, Mace E, Henry R, Jordan D (2019) Exploring and exploiting pan-genomics for crop improvement. Mol Plant 12(2):156–169

Tettelin H, Masignani V, Cieslewicz MJ, DonatiC MD et al (2005) Genome analysis of multiple pathogenic isolates of Streptococcus agalactiae: implications for the microbial "pan-genome." Proc Natl Acad Sci USA 102(39):13950–13955

Wang H, Deng XW (2018) Development of the "third-generation" hybrid rice in China. Genom Proteom Bioinformat 16(6):393–396

Wang K, Wang Z, Li F, Ye W, Wang J et al (2012) The draft genome of a diploid cotton *Gossypium raimondii*. Nat Genet 44(10):1098–1103

Wang Q, Fang L, Chen J, Hu Y, Si Z et al (2015) Genome-wide mining, characterization and development of microsatellite markers in *Gossypium* species. Sci Rep 5(1):1–10

Wang L, He S, Dia S, Sun G, Liu X et al. (2021) Alien genomic introgressions enhanced fiber strength in upland cotton (*Gossypium hirsutum* L.). Ind Crops Prod 159:113028

Wei Y, Xu Y, Lu P, Wang X, Li Z et al (2017) Salt stress responsiveness of a wild cotton species (*Gossypiumklotz schianum*) based on transcriptomic analysis. PLoS ONE 12(5):e0178313

Wendel JF (1989) New World tetraploid cottons contain Old World cytoplasm. Proc Natl Acad Sci USA 86(11):4132–4136

Wu H, Zheng L, Qanmber G, Guo M, Wang Z, Yang Z (2021) Response of phytohormone mediated plant homeodomain (PHD) family to abiotic stress in upland cotton (*Gossypium hirsutum* spp.). BMC Plant Biol 21(1):1–20

Zhenglan L, Ruqin J, Wennan Z, Jianxing H, Chuanwei S et al (2002) Creation of the technique of interspecific hybridization for breeding in cotton. Sci China Sr C Life Sci 45(3):331–336

Zhong H, Liu S, Meng X, Sun T, Deng Y et al (2021) Uncovering the genetic mechanisms regulating panicle architecture in rice with GPWAS and GWAS. BMC Genom 22(1):1–13

Zhou H, Deng H, Su J, Sun Y, Jiang L, Liu Z, TangC (2020) Breeding high-yield upland cotton (*Gossypium hirsutum*) lines with high-quality fibre by mutation. Plant Breed 139(6):1201–1212

Zhu X, Sun L, Kuppu S, Hu R, Mishra N et al (2018) The yield difference between wild-type cotton and transgenic cotton that expresses IPT depends on when water-deficit stress is applied. Sci Rep 8(1):1–11

Chapter 5
Conventional and Molecular Interventions for Abiotic Stress Resistance in Floricultural Crops

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Abstract Floriculture crops hold an important economic place in the horticulture industry. However, abiotic stresses (e.g. salt, drought, temperature) cause huge quality and quantity losses in these crops. A series of adaptive mechanisms are triggered at the physiological, biochemical and molecular levels to overcome stress and enhance survival. For instance, plants undergo growth reduction, alteration in leaf anatomy, abnormal shoot to root ratio, shrinkage of leaves to reduce water loss etc. Also, the past decades have witnessed a plethora of information underlying genetic basis of stress adaptation in floriculture crops. Implementation of physiological and omics approaches have resulted in the identification of several genes and regulatory networks imparting abiotic stress resilience. Moreover, genome editing tools like CRISPR/Cas9 offers ample opportunities for efficient and precise genome modifications, thus helpful in generating abiotic stress resistant floriculture crops. This book chapter provides an overview on abiotic stress responses in floriculture crops, and discusses various conventional and molecular breeding approaches to develop abiotic stress resilient cultivars.

Keywords Abiotic stresses · Climate change · Genetic engineering · Molecular breeding · Ornamental crops

5.1 Introduction

Ornamental plants have a great contribution in improving environment and the quality of life beside their aesthetic importance (Savé 2009). However, the major concern of ornamental plants growers and consumers is the quality and sustainability of their produce. However, several factors such as genotype, environmental conditions and management of cultivation determine the quality and yield of ornamental plants. Being sessile in nature, plants face variety of environmental stresses including drought, flooding, temperatures extremes, salinity, heavy metal, nutrient

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insufficiency, and variable light intensities, including ultraviolet radiation. Floriculture crops are grown under greenhouses as well as in the fields, so they have number of chances to meet abiotic stressors such as nutrient or water stress. These stresses affect crops during production or post-production, and commonly lead to poor quality. Adaptation capacity to various abiotic stresses, governs the survival of plants. Few plants can develop resilience against abiotic stresses by implying different approaches to adapt to or by escaping of the harmful effects of abiotic stresses. Abiotic stresses, in mild form boost up the defence system of plants and thus, can be beneficial for crops as it leads to accumulation of few metabolites/bioactive compounds, which ultimately improves the quality of produce during pre-harvest and postharvest times. And good quality produce of ornamentals, in contrast, severe abiotic stresses deteriorate plant growth and development due to various physiological and biochemical processes in plants and ultimately lead to huge loss in crop productivity (Toscano et al. 2019). However, due to climate change/adversities it is anticipated that there will be in inadequacy of fresh water and this will ultimately intensify the effects of abiotic stresses. Therefore, it becomes mandatory to develop cultivars that are resilient to abiotic stresses. So far, several efforts have been made to generate abiotic stress resistant crop varieties. Previously, conventional breeding methods such as phenotypic selection in target environments was used to develop tolerant varieties (Driedonks et al. 2016). With the adoption of marker assisted selection (MAS) techniques, molecular markers become the efficient tool for MAS in crop breeding for traits like abiotic stress tolerance (Younis et al. 2020). Moreover, molecular studies have a great contribution in unravelling the underlying mechanism behind abiotic stress tolerance. Such studies have helped to identify genes behind stress tolerance or susceptibility. Identification of genes itself is a big deal as it offers the opportunity to generate abiotic stress resistant varieties via genetic engineering. Furthermore, recently advancement in genome editing tools have been proved revolutionary in breeding of stress resilient varieties, by enabling gene editing efficiently and in a precise manner (Zong et al. 2017a, b). This newly emerged technology has lot of potential for the development crops of desired phenotype or genetic makeup by targeting important genes involved in abiotic stress tolerance (Klap et al. 2017).

5.2 Abiotic Stresses in Floricultural Crops

To meet the commercial global demand of floriculture crops, it has become highly desirable to cultivars that can tolerate variety of environmental stresses. Among different abiotic factors, soil salinity is a global problem that restrict growth and impact negatively on the crop productivity (Caverzan et al. 2016). High salinity is responsible for disturbing ionic and osmotic balance in plants. Majority of plants cannot tolerate salt stress and show impaired growth and development characteristics such as poor germination of seeds and seedling development, reduced vegetative and reproductive growth (Guo et al. 2018). Such morphological changes occur as a result of disturbance in photosynthesis, osmotic and ionic imbalance, oxidative stress and

scarcity of mineral supply (Feng et al. 2014). Under salt stress, various ornamental plants showed variety of symptoms such as reduction in fresh and dry weight of aerial parts, decreased leaf area, stunted plant height and decline in quality and quantity of flowers (Quist et al. 1999). In few plants, salinity also lead to the reduction in root growth which is generally caused by increased ionic toxicity in soil (Bañón et al. 2012). It was reported that due to saline conditions in soil (4 dS $^{-1}$ EC) plants of *Phlomis purpurea* showed reduced root dry weight. Similarly, garden roses (*Rosa* × *hybrida* L.) were also found be sensitive and showed reduced growth symptoms under saline conditions of above 3.0 dS m^{-1} electrical conductivity (EC). However, few selected rose cultivars have been found resistant to salt stress and showed no symptoms of poor growth and quality even at EC of 3.5 dS m^{-1} (Cabrera 2003; Niu et al. 2008).

Other environmental stresses such as water stress (also referred as drought) and temperature extremes are also considered destructive for plant growth and development in the same way as salinity (Morales et al. 2013). In the present scenario, with the increasing temperature and CO_2 levels, climate has changed globally which ultimately affects the rainfall distribution. The uneven rainfall is a main consequence of drought stress. On the onset of drought stress, the first response of plants is the growth arrest. However, subsequently, under drought, plants synthesize protective compounds and perform all the required osmotic adjustments by mobilizing metabolites. Few ornamental plants have tendency to withstand the damaging effects of drought by using adaptive mechanisms based upon morphological, physiological and biochemical alterations. Generally, plants respond to drought stress by exhibiting few morphological alterations such as stunted shoot length and reduced leaf growth. Such phenotypic alterations affect the visual appearance and ornamental value negatively. Under water stress, *antirhinum majus* cv. Butterfly showed reduced plant growth parameters (leaves and shoots growth, and flowers quantity) due to alteration in plant nutritional status (Asrar et al. 2012) On studying the effect of drought on sunflower, it was found that water stress has caused severe reduction in sunflower seedling growth (Andrade et al. 2013). Moreover, poinsettia (*Euphorbia pulcherrima*) with reducd plant height and leaf area, also indicated similar effects of drought stress (Nackley et al. 2020) Several other studies carried out on ornamental plants has confirmed that drought stress has visible harmful effects on yield and quality of crops (Toscano et al. 2014; Fita et al. 2015).

Likewise, temperature stress also causes bad effects on plants by retarding growth and development. This stress leads to seed germination inhibition, retardation of growth, alteration in phenology, yield reduction, altered photosynthesis. On the basis of range of temperature, this stress can be of different kinds that includes high temperature and low temperature (chilling or freezing). Plants cope with adverse high temperature stress by regulating physiological, biochemical and molecular mechanisms including proteins, structural and regulatory genes such as stress related transcription factors, antioxidants, synthesis of metabolites/protective substances and by altering composition of membrane lipids. Oxidative stress due to the generation of reactive oxygen species (ROS), is one of the main consequences of heat stress. Being another form of temperature stress, cold stress is as serious as heat stress and

a major threat to the crop sustainability. Most common morphological symptoms of cold stress include low germination rate, reduced growth of seedlings, chlorosis, reduced leaf area and necrosis. Under temperate conditions, usually plants come across to chilling and freezing stress, that are very injurious to plants. Sub lethal exposure of cold temperature (chilling) results in freezing tolerance and the process is termed as cold acclimation. However, many floriculture crops are unable to acclimate to cold temperature which results in growth and quality losses. Lily is one of the most important bulbous floriculture crops but found to be sensitive to cold stress. However, its wild relative named as *Lilium lancifolium* have an ability to acclimate to low temperatures up to −35 °C (Wang et al. 2014). This suggests that a distinct molecular mechanisms come into play to impart cold resistance offering candidate genes for genetic engineering techniques to enhance cold tolerance in plants (Yong et al. 2018).

5.3 Different Approaches to Alleviate Abiotic Stress in Floricultural Crops

Due to the popularity of cut flowers, use in gardening and for landscaping purposes, floriculture crops hold a significant place within the horticultural industry. Moreover, the estimate of total turnover of the global floriculture market is 300 billion USD (Azadi et al. 2016). Looking at the high commercial value of floriculture crops, it is therefore become imperative goal of the breeders for the breeding of new ornamental varieties well adapted to abiotic stresses. Successful breeding of climate smart varieties/ cultivars of floriculture crops requires implication of different traditional and molecular breeding approaches.

Even though use of conventional breeding approaches has made a worthy contribution to develop ornamental crops with improved tolerance against abiotic stress, but still, to accomplish this challenging the goal of generating climate smart crops, it becomes highly important to explore more capable and modern technologies (Driedonks et al. 2016). Although, to support the conventional breeding programs, molecular breeding involving use of molecular markers are available, but this approach come with its own limitations such as flow of undesirable characters from the donor parent along with the quantitative trait loci (QTLs) involved in stress tolerance. To overcome such limitations, use of genetic engineering for the transfer of desired gene for conferring abiotic stress tolerance is an attractive option. Moreover, the availability of highly precise and efficient gene editing tools such as clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) offers great advantage over preliminary approaches for improvement of crops against several abiotic factors in a more targeted and precise manner (Dalla Costa et al. 2017).

5.3.1 Conventional Breeding

Ornamental Crops are affected by various kind of biotic (insect, disease, and virus) and abiotic (temperature, drought, or salinity) stress and their continual cultivation rely on steady breeding programs for resistance. Among various causes of crop losses, abiotic stresses hold a promising position. Breeding methods like hybridization (inter-specific, inter-generic) and mutagenesis (in vitro or in vivo) have been proven effective ways for getting better ornamentals plants with increased abiotic stress tolerance. Conventional breeding means improving the existing gene pool of the crop for its better survivability. The first step involved in the conventional breeding method is selection of parents having desired traits viz., resistance/tolerance towards harsh environmental conditions to produce offspring with desirable traits. There are variety of breeding methods available, whose applicability depends upon the nature of crops. For self-pollinating crops mass selection, pure line selection, pedigree analysis, single seed descent, bulk population, backcrossing, multiline, composite and heterosis are different available breeding methods. However, for cross-pollinating species breeding methods like mass selection, recurrent selection, and heterosis etc. exists. In the case of ornamental crops whole market value depends upon its aesthetics therefore it is important to have cultivars with good genetics. Breeding tolerant/resistant cultivars towards abiotic stress offer an economical way out to grow a crop with minimum inputs and ensure better market value. For any breeding program to be successful, the availability of genetic variation in the form of wild germplasm and its successful transfer to the next generation is required.

5.3.1.1 Hybridization

Heterosis term was first time coined by Shull in 1914. It explains the phenomenon superiority of F1 generation over its both parents for growth, yield, earliness, growth vigour and improved biotic and abiotic stress resistance etc. As a breeding technique in plants, heterosis for biotic and abiotic stresses is very much valued. Hybridization (interspecific and intergeneric) has proven a promising approach in some crops to develop abiotic stress tolerance varieties by using their tolerant or resistant wild relatives in a breeding programme (Cattivelli et al. 2008). For example, an intergeneric hybridization between *Lolium perenne* (perennial ryegrass) and *Festuca mairei* (Atlas fescue) resulted in F1 with improved drought tolerance (Wang and Bughrara 2008). Interspecific hybrids with drought tolerance have been developed by crossses between Chrysanthemum grandiflorum (Ramat.) Kitamura and C. indicum (L.) Des Moul. (Sun et al. 2010). By using the ovary rescue technique cold tolerant interspecific hybrids were obtained between *Dendrenthema morifolium* x *Dendrenthema nankingense* (Cheng et al. 2010a, b). Likewise, through embryo rescue technique an intergeneric hybrid with improved salt tolerance was developed by a cross between *Chrysanthemum morifolium* X *Artemisia japonica* (Zhu et al. 2013a). The rose species *R. kordesii* and *R. rugosa* have been used as parents to develop hybrids

with enhanced frost resistance (Svejda 1977; 1979). Canadian Explorer and the Parkland rose are also known for their frost tolerance trait whereas, Delhi Princess is an Indian variety developed by Dr. B. P. Pal has been recognized as a high temperature tolerant (Swarup 1988).

5.3.1.2 Mutation Breeding

The introduction of mutation is highly potential way to create new variability in a population. Mutation breeding comes in handy in creating varieties that are desirable like tolerance against stress when the natural resistant source is missing in any population. It has been extensively used in the case of ornamental crops for various floral attributes such as flower pigment modification, flower structure and size, leaf growth characteristics, and chlorophyll variegation, as there is an endless demand for new floral traits in the floriculture industry (Ibrahim et al. 2018). As mutation involves genetic change which leads to heritable changes in the visual appearance of a plant This term was first time used by Hugo de Vries in 1900 by observing phenotypic changes in primrose due to genetic mutations. The individual with genetic variation is known as a mutant. Mutations are mostly lethal to the individual who carry them except for the frequency of beneficial mutation as low as 0.1% (Patil and Patil 2009). Mutations are of two kinds including natural mutation/spontaneous mutation (occurring naturally by the environment) and induced mutations or man-made mutation by using physical and chemical mutagens. It is necessary to optimize mutagen doses for every crop prior to treatment. For physical mutagens, lethal dose 50 (LD_{50}) is standardized before commercial use. LD_{50} is the amount of mutagen, given at once which causes 50% mortality of the treated individuals. For mutagen application different plant parts can be used are seed, seedling, cutting or any other plant or in vitro plantlets depending upon crop and mutagen used. For e.g. in chrysanthemum mostly tip cuttings are treated with chemical mutagens and in case of marigold seeds are treated with gamma rays. The mutation is a phenomenon occurring at the cell, tissue, or organism level. Its success depends upon the use of proper dosage of mutagen and frequency of mutation. Mutation results are usually confirmed on the basis of evaluation of putative mutants under controlled and replicated trials (Oladosu et al. 2016). Mutation breeding is time-saving technique as variability can be created in the shortest span. The tolerance of a plant to any stress was evaluated by its ability to maintain its quality or visual appearance and quantity in term of crop yield. The chrysanthemum (*Chrysanthemum morifolium* Ramat.) variant E2 variant was developed through in vitro mutagenesis using ethyl methane sulphonate (EMS) mutagen showed enhanced salt tolerance as compared to the control. It was found that upon mutation E2 variant exhibited increased levels of carotenoids and activity of various antioxidant enzymes. Such enzymes have ability to protect plant against saline conditions by preventing damage to the plasma membrane by reactive oxygen species (Hossain et al. 2006). In addition, the induction of polyploidy has been reported as an effective way to ameliorate abiotic stress in ornamental crop. A tetraploid *Dendranthema nankingense* was obtained by the colchicine treatment using nodal

explants under in vitro conditions. This mutant exhibited elevated drought and salt bearing ability by overcoming oxidative stress, maintaining proper osmotic balance and increased pigments such as chlorophylls (Liu et al. 2011).

5.3.1.3 Recurrent Selection

Due to changing climate and increasing environmental adversities there's persistent demand for new cultivars with favourable gene frequencies. The most common choice is the backcrossing of breeding variant i.e. recurrent selection, in which performance selection is made between successive segregating generations.

It helps in population enhancement but doesn't involve any new variety development. This is a cyclic selection for enhancing the changes of desirable/favourable genes frequencies in a breeding population (Ramalho et al. 2005) or it's a variant of backcross where selection for better type is exercised in segregating generations. This system is most suited to cross pollinated crops, and was first time used in maize (Bolanos and Edmeades 1993). The concept of recurrent selection was introduced by Hayes and Garber in 1919, and East and Jones in 1920 and the method was described by Jenkins in 1940 however, the term recurrent selection was first given by Hull (1945). This system can be used in one (intra-population enhancement) or two populations (inter-population enhancement) to advance the combining capability. Recurrent selection can be employed to a lesser extent if heterozygous base population and cytoplasmic male sterility (CMS) system are present.

5.3.1.4 Multiple Lines Breeding Approaches

Multiline lines are the mixture of several isogenic lines having analogous agronomic characteristics but along with different genes for complete resistance against stress. To make a variety marketable successful, a resistant variety is not sufficient. However, along with resistance characters all other agronomical features (high yield, agronomic performance, and vase life) should also be present. Thus, multiline parentage is a useful tool to develop composite verities. But this system is still under-utilized in the case of ornamental crops (Table 5.1).

5.3.2 Molecular Approaches

Ornamental plants have major applicability in cosmetic, perfume as well as food industries and play an essential role in improving the human environment and national economy. The worth of ornamental plants is based on their phenotypical characters such as flower shape, its color, size, number, and postharvest life. Environmental or abiotic factors (drought, salinity, high temperature, cold, metal ions) can adversely

Table 5.1 Status of abiotic stress management in floriculture crops through conventional approaches

Sr. No	Crop	Cross	Resistant/tolerant	References
1	Chrysanthemum	*Chrysanthemum grandiflorum* x *Chrysanthemum indicum*	Highly drought tolerant	Sun et al. (2010)
2		*Dendranthema morifolium* x *Dendranthema nankingense*	Highly cold tolerant	Cheng et al. (2010)
3		*Chrysanthemum morifolium* x *Ajania przewalskii*	High tolerance drought	Deng et al. (2011)
4		*Chrysanthemum morifolium* X *Artemisia japonica*	High tolerance salt	Zhu et al. (2013b)
5	Rose	*R. kordesii* x DO8 *R. kordesii* x G12	Cold hardiness	Svejda (1977)
6		Manetti	Salt tolerance	Cabrera et al. (2009)
7		Belinda's Dream and Climbing Pinkie	Salt tolerance	Cai et al. (2014a, b)
8		M4-4, J06-20-14-3 (tolerant) × 97/7-2, Red Fairy, Sweet Chariot, Vineyard Song, Old Blush, and Little Chief (Sensitive)	Heat tolerance	Liang et al. (2017a, b)
9		*R. yesterday* x *R. wichurana*	Drought tolerant	De Dauw et al. (2013)
10	Zinnia	Dreamland Red	Drought tolerance	Riaz et al. (2013)
11	Ornamental crops	Salvia > Dahlia ≥ Impatiens > Pentas	Dehydration tolerance	Auge et al. (2003)
12	Ornamental grasses	Bermuda grass, bahia grass, and buffalo grass (resistant) Zoysia grass and tall fescue (sensitive)	Drought	Beard and Sifers (1997)
13	Bedding plant	Geranium (*Pelargonium hortorum* L. H. Bailey) > Impatiens (*Impatiens walleriana* Hook)	Drought tolerance	Chylinski et al. (2007)
14	Herbaceous ornamental annuals	Petunia and Glandularia	Drought tolerance	Henson et al. (2006)
15	Turf grass	Khabbal (more tolerant) Dacca and Fine Dacca (less tolerant	Drought tolerance	Riaz et al. (2010)
16		*Festuca ovina* > *Lolium perenne* = *Poa compressa* > *Poa pratensis* = *Koeleria glauca* > *Festuca rubra* subsp. Rubra > *Festuca rubra* subsp. Commutata > *Agrostis capillaris* > *Deschampsia caespitosa*	Drought tolerance	Kanapeckas et al. (2008)

(continued)

Table 5.1 (continued)

Sr. No	Crop	Cross	Resistant/tolerant	References
17	Geranium	Strawberry sizzle and violet	Heat tolerance	https://bexar-tx.tamu.edu/homehort/archives-of-weekly-articles-davids-plant-of-the-week/the-first-truly-heat-tolerant-geraniums-in-the-world/

affect the morphology and physiology resulting in drastic impact on quality, productivity and commercial value of plants. Therefore, various traditional and modern breeding approaches have been developed for improving abiotic stress tolerance in ornamental crops. Conventional breeding directly relies on the phenotypic observations lacking any information on associated genes. However, modern molecular breeding approaches offers rapid and accurate process in identifying trait of interest at any growth stage without wasting time, space and valuable resources.

5.3.2.1 Molecular Breeding

In the last few years, developing abiotic stress tolerant varieties in ornamental crops have gained significant attention. Development of DNA-based markers techniques render to use molecular marker-assisted selection (MAS) in current breeding methods. This approach allows the selection of target traits indirectly without paying attention to abiotic factors. These molecular breeding approaches has been widely used in field crops e.g. rice, wheat, maize but there are very few reports available in ornamental crops. Chrysanthemum, an important cut flower in the floriculture industry known for its wide range of colour, forms, good keeping and transportation quality but found to be sensitive for low temperature, water stress and salinity (Hong et al. 2006). Genetic architecture of drought tolerance in *Chrysanthemum morifolium* Ramat. has been deciphered by GWAS (genome-wide association study) which is based upon the 707 informative molecular markers (Li et al. 2016). Drought tolerance level has been quantified by measuring the average subordinate function value (ASFV) that integrates the wilting index as well as survival rate. Several cultivars with four favourable alleles (*E11M24-9, E3M2-8, E1M5-5* and *EST-SSR34-3*) have been recognized as potential donor for improvement of drought tolerance (Li et al. 2018). First linkage map from a bi-parental cross in chrysanthemum was produced

by random amplified length polymorphism (RAPD), amplified fragment length polymorphism (AFLP) and inter-simple sequence repeat (ISSR) markers (Zhang et al. 2019). In *Dianthus caryophyllus*, using genome wide identification approach Heat shock transcription factors have been characterized, resulted into the identification of 17 non-redundant (*Hsfs*). Upon the analysis of gene structure and promoter, various cis-acting elements (*MYB*, *STRE* and *ABRE* binding sites) and heat and drought responsive elements (*DcaHsf-A1, A2a, A9a, B2a*, *B3a*) were found to be involved in protection of plant damage from abiotic stresses (Li et al. 2019). Weighted gene co-expression network analysis (WGCNA) was performed in a cultivated ornamental plant named as *Rosa chinensis* 'Old Blush'. It was found that the constructed co-expression network was in close association with the physiological characters of drought response. About 42 transcriptional factors (*NACs, WRKYs, ARFs*) have been identified and used for the improvement of drought tolerance in rose cultivars (Jia et al. 2021).

5.3.2.2 Genetic Engineering

Genetic engineering approach offers numerous options for the improvement of abiotic stress in ornamentals. This technology employs the use of variety of structural genes which are involved in the synthesis of osmo-protectants and heat as well as cold shock proteins, along with regulatory genes including transcription factors like *DREB1*, *MYB*, *MAPK*, *WRKY* gene family etc. Recombinant DNA technology allows the transfer of appropriate genes of interest to the susceptible plants for development of cultivars with desired/valuable traits. Genetic engineering offers advantage over conventional breeding that involves mixing of thousands of genes randomly, present in resistant and susceptible plants. Moreover, for development of new varieties, traditional methods seem time-consuming and difficult process due to problem of sterility in crops like orchids (Da Silva et al. 2011). Therefore, genetic engineering provides an alternative platform for the varietal improvement. The key target genes for imparting drought stress resistance includes genes of ABA biosynthesis and signalling pathway. Under water or drought stress, concentration of ABA increases and leads to closure of stomata to minimize the transpiration Prolonged exposure to osmotic stress due to dehydration activates osmotic-responsive genes and build-up of organic solutes also like mannitol, betaine, proline, glycine etc. referred as osmo-protectants. Drought stress tolerance was achieved in petunia by overexpressing a *p5cs* gene (pyrroline-5-carboxylate synthetase) involved in proline biosynthesis (Yamada et al. 2005). Another important class of proteins referred as LEA (late embryogenesis abundant) proteins are involved in protecting the cells from stress. Thus genes expressing LEA proteins are also good candidates providing desiccation tolerance in plants. Moreover, water transporters proteins such as aquaporins, antioxidant enzymes like peroxidase, superoxide dismutase and catalase also acts as regulatory molecules in combating abiotic stress. Without having any detrimental effects on growth and development, drought tolerance in petunia has been achieved by the heterologous expression of *LeNCED1* (9-cis-epoxycarotenoid dioxygenase

gene) gene under the control of a stress-specific promoter (*rd29A*) (Estrada-Melo et al. 2015). In a similar study, *GLDH* gene (L-galactono-1,4-lactone dehydrogenase) isolated from apple was overexpessed in *Lilium davidii* var. *unicolor.* This gene transfer lead to considerably amplified ascorbic acid quantity and conferred abiotic stress resistance (Shi et al. 2012). Generally, transcription factors (*TFs*) are known to regulate the expression of structural genes in response to any physical and environmental stimuli. There are several TFs which have a distinct role in imparting abiotic stress tolerance. Among them *WRKY* transcription factors (*TFs*) have a defined role in regulating abiotic stress in plants positively and negatively (few). Over expression of a transcription factor, *CmWRKY17* isolated from *Chrysanthemum morifolium* conferred improved salinity tolerance in chrysanthemum, and this by downregulating gene expression of several genes such as *AtRD29, AtDREB2B, AtSOS1, AtSOS2 and AtSOS3* involved in stress responses (Li et al. 2015). The overexpression of *CmWRKY1 TF* in chrysanthemum cultivar 'Jinba' has resulted in the transformed plants that showed improved drought stress tolerance as compared to control plants. This tolerance in transgenic plants was achieved due to increased levels of expression of stress responsive genes of ABA signalling pathway such as *DREB1A, ABI1, ABI2, MYB2 and RAB18* (Fan et al. 2016). However, to overcome the negative effects of heat stress on plant growth, one of most promising strategy is the cver-expression of heat shock proteins (*HSPs*) in plants. As molecular chaperons, *HSPs* are involved in proper protein folding and assembly. Such proteins offer stability to various integral proteins under high temperature stress (Boston et al. 1996). In study conducted on chrysanthemum, over-expression of *CgHSP70* gene conferred heat tolerance. As a result, it was found that transgenic lines exhibited increased peroxidase activity and higher proline content. As a crucial osmoprotectant, proline has a protective role against the damaging effects of heat stress. Thus increased proline content in transgenic plants clearly defines their ability to cope up with heat stress in comparison to control plants. Introduction of zinc finger transcription factor gene BBX24 in *Chrysanthemum morifolium* influenced the flowering time and enhanced freezing as well as drought stress tolerance. This transcription factor is known to have a role in regulation of photoperiodic pathways Thus improved tolerance against abiotic stresses in chrysanthemum is as a result of gibberellin biosynthesis modulation (Yang et al. 2014). Freezing tolerance in China rose has been enhanced by incorporating *MtDREB1C* gene isolated from *Medicago trancatula* (Chen et al. 2010). Cold stress tolerant varieties of chrysanthemum with least effects on plant growth even during winter, have been developed by transfer of a *AtDREB1A* gene (Hong et al. 2006). Transfer of *CBF3* gene from *Arabidopsis thaliana* into *Petunia* × *hybrida* (petunia) resulted in enhanced frost tolerance (Warner 2011). A similar outcome of abiotic stress/drought tolerance was attained in rose by the overexpression of a transcription factor (*RhNAC3)* isolated from *Rosa hybrida* (Jiang et al. 2014). Therefore, taking into consideration above quoted examples, authors can suggest that genetic engineering offers unmatched platform for abiotic stress tolerance, particularly in ornamental crops coupled with intact economical trait.

5.3.2.3 RNAi/Antisense RNA Technology

RNAi is a RNA based gene silencing technique. It is naturally occurring defence mechanism in the host developed against the viral messenger-RNAs where small RNA interrupt the gene expression and interfere with the translation of target mRNA transcript. This tool has been previously applied in few ornamental crops for manipulation of gene expression. *PhERF2*-RNAi lines of petunia has demonstrated role of *PhERF2* (An ethylene–responsive element binding factor) as a negative regulator in imparting waterlogging tolerance (Yin et al. 2019). Similarly, in case of *Chrysanthemum morifolium*, the *CmNF-YB8*- RNAi lines showed resistance against drought as compared to overexpression lines of *CmNF-YB8*. The RNAi lines lead to the lead to the alteration of stomatal opening rate and increase in the cuticle thickness of leaf epidermis to minimize transpiration rate (Wang et al. 2021). Similarly, zinc figure protein *BBX19* interacts with the *ABF3* in *CmBBX19*—suppression lines shows increase drought tolerance through ABA-dependent pathways as compared to control plants (Xu et al. 2020).

5.3.2.4 Gene Editing

Among recently developed tools for crop improvement, gene editing is one of the most important technique of trait improvement that has the potential to meet the global demand of floriculture crops. Several genome editing techniques like transcriptional activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) have been used for genetic manipulations in plants. However, CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeats/ CRISPR-associated protein 9) technique is getting highly popular due to its convenient use, simpler design and higher efficiency for targeting single and multiple genes. Although at present, no report of using CRISPR/Cas9 approach for imparting abiotic stress resistance, is available in ornamental crops. However, this approach has tremendous potential and scope in improving tolerance against abiotic stresses in floriculture crops. Availability of tissue culture and efficient genetic transformation protocols is a prime requirement for utilizing the application of CRISPR Cas9 system in ornamental plants. Moreover, for successful implementation of this versatile approach in ornamental crops, knowledge of structure and functionality of the genes and genome is required. Till date, genome and transcriptome sequences are available in numerous ornamentals including carnation (Jo et al. 2015), *Lagerstroemia indica* (Wang et al. 2015), Tulip (Miao et al. 2016; He et al. 2019), orchid (Niu et al. 2016), rose (Moghaddam et al. 2014; Diaz-Lara et al. 2020; Chandran et al. 2021), Gerbera (Fu et al. 2016; Bhattarai et al. 2020) etc. For the applicability of CRISPR Cas9 system for gene editing in ornamental species having high ploidy level and genome size, the information of sequence of genome or genes (to be targeted) can be taken from the genome sequence available in its wild relative. The availability of genome or gene sequence will help in the identification of potential targets involved in abiotic stress signalling pathways (SOS1, SOS3, CBFs, DREBs, MAPK, WRKY, NACs, bZIPs, MYBs) and

will also provide fundamental genetic information for the genome editing through CRISPR/Cas9 to develop new abiotic resistant ornamental varieties (Table 5.2).

5.4 Conclusion

The increasing popularity of floriculture crops worldwide has resulted in high demand of elite cultivars in the floriculture industry. Besides, breeding for new cultivars with improved floral attributes such as flower colour, shape, size, scent and vase life, another important target include generation of abiotic stress tolerant floriculture crops. Abiotic stresses such as drought, salinity, temperature, etc. are known to cause detrimental effects on phenotypic and yield (number of flowers) traits. The deterioration of quality and quantity traits lower the economic value, thus negatively affecting floriculture market. Therefore, there is an urgent need to develop and introduce new ornamental varieties that are resistant to abiotic stresses. Although attempts have been made to create abiotic stress tolerant varieties through conventional breeding tools. However, the usage of conventional breeding approaches gets restricted due to high ploidy level, heterozygosity, large genome size, and sterility problem in ornamentals. Also, conventional breeding approaches are time-consuming. Furthermore, the creation of abiotic resistant ornamental varieties is lagging behind due to lack of understanding of stress-induced gene regulatory pathways. The implementation of next-generation sequencing complemented with other omics technologies can help in the better understanding of gene regulatory networks underpinning abiotic stress response. The knowledge gained at the level of genes, promoters, small RNAs, epigenetic modifications, etc. can help in the creation of abiotic stress tolerant floriculture crops by using CRISPR/Cas9 system. CRISPR/Cas9 has multiple advantages such as efficiency, precision and multiple gene targeting and has previously been applied in model floriculture crops. Thus, it holds a great potential to develop abiotic stress tolerance in commercially important floriculture crops.

Table 5.2 Status of abiotic stress management in floriculture crops through molecular approaches

S.No	Crop name	Targeted resistance	Target gene (s)	Molecular approach	Reference
1	Chrysanthemum	Salinity stress	*CmWRKY17*	Gene transfer	Li et al. (2015)
		Enhanced salt stress tolerance and water deficiency	35S:*DREB1A* and *rd29A*:*DREB1A*	Gene transfer	Hong et al. (2006)
		Salt stress tolerance	*CcSOS1* encoding plasma Na+/H+ antiporter	Gene transfer	An et al. (2014)
		Salt and drought tolerance	*ClCBF1*	Over expression	Gao et al. (2018)
		Heat, drought and salinity stress	CgHSP70 and DgNAC1	Over expression	Song et al. (2014); Wang et al. (2017a, b)
		Drought tolerance	CmBBX19	Over expression	Xu et al. (2020)
		Salt resistance	DgWRKY5	Gene transfer	Liang et al. (2017a, b)
		Drought tolerance	CmPLDα (phospholipase)	Over expression	Zhai et al. (2021)
		Salt tolerance	DgWRKY2	Over expression	He et al. (2018)
		Saline, alkaline, and drought resistance	ClNAC9	Over expression	Dong et al. (2018)
2	Rose	Freezing and drought tolerance	*RcXET* and *MtDREBIC*	Gene transfer	Chen et al. (2016)
		Enhanced salinity tolerance	*AtDREB2A-CA*	Gene transfer	Josine et al. (2015)
3	Petunia	Frost tolerance	*AtCBF3*	Gene transfer	Warner (2011)
		Salt and drought tolerance	AtNHX1	Over expression	Xu et al. (2009)

(continued)

Table 5.2 (continued)

S.No	Crop name	Targeted resistance	Target gene (s)	Molecular approach	Reference
4	*Dendrathema grandiflorum*	Heat stress tolerance	*AtDREB1A*	Over expression	Hong et al. (2009)
5	*Lilium davidii* var. *unicolor*	Abiotic stress	GLDH	Over expression	Shi et al. (2012)
6	Water lily	Cold stress tolerance	CodA	Pollen tube transformation	Yu et al. (2018)
7	Orchid *(Phalaenopsis amabilis*	Cold stress tolerance	LTP (lipid transfer protein)	*Agrobacteriu m* mediated gene transfer	Qin et al. (2011)
8	Lotus	Salt tolerance	NnCIPKgt(calcineurin B-like protein kinase)	Gene transfer	Liu et al. (2014)

References

An J, Song A, Guan Z, Jiang J, Chen F, Lou W, Fang W, Liu Z, Chen S (2014) The over-expression of *Chrysanthemum crassum CcSOS1* improves the salinity tolerance of chrysanthemum. Mol Bio Rep 41(6):4155–4162

Andrade A, Vigliocco A, Alemano S, Llanes A, Abdala G (2013) Comparative morpho-biochemical responses of sunflower lines sensitive and tolerant to water stress. Amer J Plant Sci 4:156–167

Asrar AA, Abdel-Fattah GM, Elhindi KM (2012) Improving growth, flower yield, and water relations of snapdragon (*Antirhinum majus* L.) plants grown under well-watered and water-stress conditions using arbuscular mycorrhizal fungi. Photosynthetica 50:305–316

Auge RM, Stodola AJW, Moore JL, Klingeman WE, Duan X (2003) Comparative dehydration tolerance of foliage of several ornamental crops. Sci Hort 98:511–516

Azadi P, Bagheri H, Nalousi AM, Nazari F, Chandler SF (2016) Current status and biotechnological advances in genetic engineering of ornamental plants. Biotechnol Adv 34(6):1073–1090

Beard JB, Sifers SI (1997) Genetic diversity in dehydration avoidance and drought resistance within the Cynodon and Zoysia species. Intl Turf Grass Soc Res J 8:603–610

Bhattarai K, Conesa A, Xiao S et al (2020) Sequencing and analysis of gerbera daisy leaf transcriptomes reveal disease resistance and susceptibility genes differentially expressed and associated with powdery mildew resistance. BMC Plant Biol 20:539

Bolanos J, Edmeades GO (1993) Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass, and radiation utilization. Field Crops Res 31(3):233–252

Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. Plant Mol Biol 32:191–222

Cabrera RI (2003) Demarcating salinity tolerance in greenhouse roses. Acta Hort 609:51–57

Cabrera RI, Solis-Perez AR, Sloan JJ (2009) Greenhouse rose yield and ion accumulation responses to salt stress as modulated by rootstock selection. Hort Sci 44:2000–2008

Cai X, Niu G, Starman T, Hall C (2014a) Response of six garden roses (*Rosa* × *hybrida* L.) to salt stress Sci Hort (Amsterdam) 168:27–32

Cai X, Sun Y, Starman T, Hall C, Niu G (2014b) Response of 18 earth-kind® rose cultivars to salt stress. Hort Sci 49:544–549

Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Mare C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res 105:1–14

Caverzan A, Casassola A, Brammer SP (2016) Antioxidant responses of wheat plants under stress. Genet Mol Biol 39:1–6

Chandran KN, Sriram S, Prakash T, Budhwar R (2021) Transcriptome changes in resistant and susceptible rose in response to powdery mildew. J Plant Phyto 169(9):556–559

Chen JR, Chen YB, Ziemianska M, Liu R, Deng ZN, Niedzwiecka-Filipiak I, Li YL, Jiao JX, Xiong XY (2016) Co-expression of *MtDREB1C* and *RcXET* enhances stress tolerance of transgenic China rose (*Rosa chinensis* Jacq.) J Plant Growth Regul 35:586–599

Chen JR. Lu JJ, Liu R, Xiong X, Wang TX, Chen SY, Guo LB, Wang HF (2010) *DREB1C* from *Medicago truncatula* enhances freezing tolerance in transgenic *M. truncatula* and China Rose (*Rosa chinensis* Jacq.). J Plant Growth Regul 60(3):199–211

Cheng X, Chen S, Chen F, Deng Y, Fang W, Tang F, Liu Z, Shao W (2010a) Creating novel chrysanthemum germplasm via interspecific hybridization and backcrossing. Euphytica 177:45–53

Cheng X, Chen S, Chen F, Fang W, Deng Y, She L (2010b) Interspecific hybrids between *Dendranthema morifolium* (Ramat) Kitamura and *D nankingense* (Nakai) Tzvel achieved using ovary rescue and their cold tolerance characteristics. Euphytica 172(1):101–108

Cheng X, Chen SM, Chen FD, Fang WM, Deng YM, She LF (2009) Interspecific hybrids between *Dendranthema morifolium* (Ramat.) Kitamura and *D. nankingense* (Nakai) Tzvel achieved using ovary rescue and their cold tolerance characteristics. Euphytica 172:101–108

Chylinski WK, Lukaszewska AJ, Kutnik K (2007) Drought response of two bedding plants. Acta Physiol Plant 29(5):399–406

Da Silva JAT, Van PT, Mii M (2011) Transgenic orchids. Sci Hort 130(4):673–680

Dalla Costa L, Malnoy M, Gribaudo I (2017) Breeding next generation tree fruits: technical and legal challenges. Hort Res 4:17067 (2017)

De Dauw K, Van Labeke MC, Leus L, Van Huylenbroeck J (2013) Drought tolerance screening of a Rosa population. Acta Hort 990:121–127

Deng Y, Chen S, Chen F, Cheng X, Zhang F (2011) The embryo rescue derived intergeneric hybrid between chrysanthemum and *Ajania przewalskii* shows enhanced cold tolerance. Plant Cell Rep 30(12):2177–2186

Diaz-Lara A, Mollov D, Golino D et al (2020) Complete genome sequence of rose virus A, the first Carla virus identified in rose. Arch Virol 165:241–244

Dong F, Huang H, Liu J, Zhang M, Zhou Y, Dai S (2018) Cloning and function analysis of *ClNAC9* from *Chrysanthemum lavandulifolium.* Can J Plant Sci 98:1265–1279

Driedonks N, Rieu I, Vriezen WH (2016) Breeding for plant heat tolerance at vegetative and reproductive stages. Plant Reprod 29:67–79

Estrada-Melo AC, Chao RMS, Jiang CZ (2015) Overexpression of an ABA biosynthesis gene using a stress-inducible promoter enhances drought resistance in petunia. Hort Res 2:15013

Fan Q, Song A, Jiang J, Zhang T, Sun H, Wang Y, Chen S, Chen F (2016) *CmWRKY1* enhances the dehydration tolerance of Chrysanthemum through the regulation of ABA-associated genes. PLoS ONE 11(3):e0150572

Feng ZT, Deng YQ, Fan H, Sun QJ, Sui N, Wang BS (2014) Effects of NaCl stress on the growth and photosynthetic characteristics of *Ulmuspumila L.* seedlings in sand culture. Photosynthetica 52:313–320

Fita A, Rodro aguez-Burruezo A, Boscaiu M, Prohens J, Vicente O, (2015) Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. Front Plant Sci 6:978

Fu Y, Esselink GD, Visser RGF, van Tuyl JM, Arens P (2016) Transcriptome analysis of gerbera hybrida including in silico confirmation of defense genes found. Front Plant Sci 7:247

Gao W, He M, Liu J, Ma X, Zhang Y, Dai S, Zhou Y (2018) Overexpression of *Chrysanthemum lavandulifolium ClCBF1* in *Chrysanthemum morifolium* 'White Snow' improves the level of salinity and drought tolerance. Plant Physiol Biochem 124:50–58

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

García-Caparrós P, Lao MT (2018) The effects of salt stress on ornamental plants and integrative cultivation practices. Sci Hortic 430–439

He L, Wu HY, Zhao Q, Wang B, Liu LQ, Zhang L (2018) Chrysanthemum *DgWRKY2* gene enhances tolerance to salt stress in transgenic chrysanthemum. Intl J Mol Sci 19:2062

He X, Li W, Zhang W, JinX SGA, Aynalem T, Xu S, Wang W (2019) Transcriptome sequencing analysis provides insights into the response to *Fusarium oxysporum* in *Lilium pumilum*. Evo Bioinform 15:1–10

Henson DY, Newman SE, Hartley DE (2006) Performance of selected herbaceous annual ornamentals grown at decreasing levels of irrigation. Hort Sci 41:1481–1486

Hong B, Ma C, Yang Y, Wang T, Yamaguchi-Shinozaki K, Gao J (2009) Over-expression of *AtDREB1A* in chrysanthemum enhances tolerance to heat stress. Plant Mol Biol 70(3):231–240

Hong B, Tong Z, Li Q (2006) Regeneration and transformation through somatic embryogenesis and determination of cold stress tolerance in ground cover Chrysanthemum cv. Fall Color Sci Agri Sin 39(7):1443–1450

Hossain Z, Mandal AKA, Datta SK, Biswas AK (2006) Development of NaCl-tolerant strain in *chrysanthemum morifolium* through in vitro mutagenesis. Plant Biol 8(4):450–461

Hull F H (1945) Recurrent selection for specific combining ability in corn Agron J 37(2):134–145

Ibrahim R, Ahmad Z, Sallah SH, Abu Hassan A, Ariffin S (2018) Mutation breeding in ornamentals. In: Van Huylen broeck J (ed) Ornamental Crops. Springer, pp 175–211

Jia X, Feng H, Bu Y, Ji N, Lyu Y, Zhao S (2021) Comparative transcriptome and weighted gene co-expression network analysis identify key transcription factors of *Rosa chinensis* 'Old Blush' after exposure to a gradual drought stress followed by recovery. Front Genet 12:690264

Jiang G, Jiang X, Lu P, Liu J, Gao J, Zhang C (2014) The rose (*Rosa hybrida*) NAC transcription factor 3 gene, RhNAC3, involved in ABA signaling pathway both in rose and Arabidopsis. PLoS ONE 9(10):e109415

Jo Y, Choi H, Cho WK (2015) Complete genome sequence of a carnation mottle virus infecting hop plants. Genome Announc 3(3):e00416-e515

Josine TL, Ji J, Wang G, Zhao Q, Yang H, Wu W (2015) AtDREB2A-CA gene over-expression in *Rosa Chinensis* Jacq. affect leaf ultrastructure response to salt stress. Intl J Agri Crop Sci 8:463–476

Kanapeckas J, Lemeziene N, Stukonis V, Tarakanovas P (2008) Drought tolerance of turfgrass genetic resources. Biologia 54:121–124

Klap C, Yeshayahou E, Bolger AM, Arazi T, Gupta SK, Shabtai S, Usadel B, Salts Y, Barg R (2017) Tomato facultative parthenocarpy results from SlAGAMOUS-LIKE 6 loss of function. Plant Biotechnol J 15:634–647

Li P, Song A, Wang L, Wang Y, Sun J, Jiang J, Chn F, Chen S (2015) Chrysanthemum *WRKY gene CmWRKY17* negatively regulates salt stress tolerance in transgenic chrysanthemum and Arabidopsis plants. Plant Cell Rep 34(8):1365–1378

Li P, Zhang F, Chen S, Jiang J, Wang H, Su J, Fang W, Guan Z, Chen F (2016) Genetic diversity, population structure and association analysis in cut chrysanthemum (*Chrysanthemum morifolium* Ramat.). Mol Gen Genom 291:1117–1125

Li W, Wan XL, Yu JY, Wang KL, Zhang J (2019) Genome-wide identification, classification and expression analysis of the *Hsf* gene family in carnation (*Dianthus caryophyllus*). Intl J Mol Sci 20(20):5233

Liang S, Wu X, Byrne D (2017a) Flower-size heritability and floral heat-shock tolerance in diploid roses. Hort Sci 52:682–685

Liang YQ, Wu HY, Wang K, Bai YZ, Liu LQ, Pan ZY, Zhang L, Jiang BB (2017b) Chrysanthemum *WRKY* gene *DgWRKY5* enhances tolerance to salt stress in transgenic chrysanthemum. Sci Rep 7:4799

Liu R, Shi H, Wang Y, Chen S, Deng J (2014) Comparative physiological analysis of lotus (*Nelum bonucifera*) cultivars in response to salt stress and cloning of *NnCIPK* genes. Sci Hort 173:29–36

Liu S, Chen S, Chen Y, Guan Z, Yin D, Chen F (2011) In vitro induced tetraploid of Dendranthema nankingense (Nakai) Tzvel shows an improved level of abiotic stress tolerance. Sci Hort 127(3):411–419

Li P, Su J, Guan Z, Fang W, Chen F, Zhang F (2018 May) Association analysis of drought tolerance in cut chrysanthemum (Chrysanthemum morifolium Ramat.) at seedling stage. 3 Biotech 8(5):226 https://doi.org/10.1007/s13205-018-1258-3

Miao Y, Zhu Z, Guo Q, Zhu Y, Yang X, Sun Y (2016) Transcriptome analysis of differentially expressed genes provides insight into stolon formation in *Tulip aedulis*. Front Plant Sci 7:409

Moghaddam H, Angelo D, Erik VN, Huylenbroeck VJ, Leen L (2014) Roses exhibit pathotype-specific resistance responses to powdery mildew. J Phyto 162(2):107–115

Morales CG, Pino MT, del Pozo A (2013) Phenological and physiological responses to drought stress and subsequent rehydration cycles in two raspberry cultivars. Sci Hort 162:239–241

Nackley LL, de Sousa EF, Pitton BJL, Sisneroz J, Oki LR (2020) Developing a water-stress index for potted Poinsettia production. Hort Sci 55:1295–1302

Niu CS, Xu Q, Zhang QG, Zhang QY, Tsai CW, Hsu LJ, Liang KC, Luo BY, Liu JZ (2016) De novo transcriptome assembly databases for the butterfly orchid *Phalaenopsis equestris*. Sci Data 3:160083

Niu G, Rodriguez DS, Aguiniga L (2008) Effect of saline water irrigation on growth and physiological responses of three rose rootstocks. Hort Sci 43(5):1479

Oladosu Y, Rafii MY, Abdullah N, Hussin GH, Ramli A, Rahim HA, Miah G, Usman M (2016) Principle and application of plant mutagenesis in crop improvement: a review. Biotechnol Biotechnol Equip 30(1):1–16

Patil SD, Patil HE (2009) Improvement of major ornamental crops through mutation breeding. Intl J Agri Sci 5(2):628–632

Qin X, Liu Y, Mao S, Li T, Wu H, Chu C, Wang Y (2011) Genetic transformation of lipid transfer protein encoding gene in *Phalaenopsis amabilis* to enhance cold resistance. Euphytica 177:33–43

Quist TM, Williams CF, Robinson ML (1999) Effects of varying water quality on growth and appearance of landscape plants. J Environ Hort 17:88–91

Ramalho MAP, Abreu AFB, Santos JB (2005) Genetic progress after four cycles of recurrent selection for yield and grain traits in common bean. Euphytica 144:23–29

Riaz A, Martin P, Riaz S, Younis A, Hameed M (2013) Comparative performance of two zinnia (Zinnia elegans) cultivars under high-temperature and limited water conditions in Pakistan. Acta Hort 980:69–78

Riaz A, Younis A, Hameed M, Kiran S (2010) Morphological and biochemical responses of turf grasses to water deficit conditions. Pak J Bot 42(5):3441–3448

Save R (2009) What is stress and how to deal with it in ornamental plants? Acta Hort 813:241–254

Shi S, Ma F, Li Y, Feng F, Shang Z (2012) Overexpression of L-galactono-1,4-lactone dehydrogenase (GLDH) in Lanzhou lily (Lilium davidii var. unicolor) via particle bombardment-mediated transformation. In Vitro Cell Dev Biol 48(1):1–6

Song A, Zhu X, Chen F, Gao H, Jiang J, Chen S (2014) A Chrysanthemum heat shock protein confers tolerance to abiotic stress. Intl J Mol Sci 15(3):5063–5078

Sun CQ, Chen FD, Teng NJ, Liu ZL, Fang WM, Hou XL (2010) Interspecific hybrids between *Chrysanthemum grandiflorum* (Ramat) Kitamura and C indicum (L) Des Moul. and their drought tolerance evaluation. Euphytica 174(1):51–60

Svejda F (1977) Breeding for improvement of flowering attributes of winter hardy Rosa kordesiiWuiff hybrids. Euphytica 26:703–708

Svejda F (1979) Inheritance of winter hardiness in roses. Euphytica 28:309–314

Swarup V (1988) Rose breeding—an overview. Indian Rose Annu 43–53

Toscano S, Ferrante A, Romano D (2019) Response of Mediterranean ornamental plants to drought stress. Sci Hort 5(1):6

Toscano S, Scuderi D, Giuffrida F, Romano D (2014) Responses of Mediterranean ornamental shrubs to drought stress and recovery. Sci Hort 178:145–153

Wang J, Yang Y, Liu X, Huang J, Wang Q, Gu J, Lu Y (2014) Transcriptome profiling of the cold response and signalling pathways in *Lilium lancifolium*. BMC Genomics 15:203

Wang JP, Bughrara SS (2008) Evaluation of drought tolerance for Atlas fescue, perennial ryegrass, and their progeny. Euphytica 164:113–122

Wang K, Zhong M, Wu YH, Bai ZY, Liang QY, Liu QL, Pan YZ, Zhang L, Jiang BB, Jia Y, Liu GL (2017a) Overexpression of a chrysanthemum transcription factor gene *DgNAC1* improves the salinity tolerance in chrysanthemum. Plant Cell Rep 6(4):571–581

Wang K, Zhong M, Wu YH, Bai ZY, Liang QY, Liu QL, Pan YZ, Zhang L, Jiang BB, Jia Y, Liu GL (2017b) Overexpression of a chrysanthemum transcription factor gene *DgNAC1* improves the salinity tolerance in chrysanthemum. Plant Cell Rep 36(4):571–581

Wang T, Wei Q, Wang Z, Liu W, Zhao X, Ma C, Gao J, Xu Y, Hong B (2021) CmNF-YB8 affects drought resistance in chrysanthemum by altering stomatal status and leaf cuticle thickness. J Integr Plant Biol PMID: 34889055

Wang X, Shi W, Rinehart T (2015) Transcriptomes that confer to plant defense against powdery mildew disease in *Lagerstroemia indica*. Intl J Genom 528395:1–12

Warner RM (2011) Genetic approaches to improve cold tolerance of petunia. https://endowment.org/wp-content/uploads/2014/03/306Warner.pdf

Xu Y, Zhao X, Aiwaili P, Mu X, Zhao M, Zhao J, Cheng L, Ma C, Gao J, Hong B (2020) A zinc finger protein *BBX19* interacts with ABF3 to affect drought tolerance negatively in chrysanthemum. Plant J 103(5):1783–1795

Xu K, Hong P, Luo L, Xia T (2009) Overexpression of *AtNHX1*, a vacuolar Na+/H+ antiporter from *Arabidopsis thaliana*, in *Petunia hybrida* enhances salt and drought Tolerance. J Plant Biol 52:453–461

Yamada M, Morishita H, Urano K, Shiozaki N, Yamaguchi-Shinozaki K, Shinozaki K, Yoshiba Y (2005) Effects of free proline accumulation in petunias under drought stress. J Exp Bot 56(417):1975–1981

Yang Y, Ma C, Xu Y, Wei Q, Imtiaz M, Lan H, Gao S, Cheng L, Wang M, Fei Z, Hong B, Gao J (2014) A zinc finger protein regulates flowering time and abiotic stress tolerance in Chrysanthemum by modulating gibberellins biosynthesis. Plant Cell 26(5):2038–2054

Yin D, Sun D, Han Z, Dian Ni1, Norris A and Jiang Z C, (2019) *PhERF2*, an ethylene-responsive element binding factor, plays an essential role in waterlogging tolerance of petunia. Hort Res 6:83

Yong YB, Li WQ, Wang JM, Zhang Y, Lu YM (2018) Identification of gene co-expression networks involved in cold resistance of *Lilium lancifolium*. Biol Plant 62(2):287–298

Younis A, Ramzan F, Ramzan Y, Zulfiqar F, Ahsan M, Lim KB (2020) Molecular Markers Improve Abiotic Stress Tolerance in Crops: a Review. Plants 9(10):1374

Yu C, Qiao G, Qiu W, Yu D, Zhou S, Shen Y, Yu G, Jiang J, Han X, Liu M, Zhang L, Chen F, Chen Y, Zhuo R (2018) Molecular breeding of water lily: engineering cold stress tolerance into tropical water lily. Hort Res 5:73

Zhai L, Zhu X, Yang S, Gu C, Liu P et al (2021) Constitutive expression of a chrysanthemum phospholipase *Dα gene* in *Chrysanthemum morifolium* enhances drought tolerance. Ornamen Plant Res 1:8

Zhang F, Chen S, Chen F, Fang W, Li F (2019) A preliminary genetic linkage map of chrysanthemum (*Chrysanthemum morifolium*) cultivars using RAPD, ISSR and AFLP markers. Sci Hort 125(3):422–428

Zhu WY, Jiang JF, Chen SM, Wang L, Xu LL, Wang HB, Li PL, Guan ZY, Chen FD (2013a) Intergeneric hybrid between Chrysanthemum morifolium and Artemisia japonicaachieved via embryo rescue shows salt tolerance. Euphytica 191(1):101–119

Zhu WY, Jiang JF, Chen SM, Wang L, Xu LL, Wang HB, Li PL, Guan ZY, Chen FD (2013b) Intergeneric hybrid between *Chrysanthemum morifolium* and Artemisia japonica achieved via embryo rescue shows salt tolerance. Euphytica 191(1):101–119

Zong Y, Wang Y, Li C, Zhang R, Chen K, Ran Y et al (2017a) Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nat Biotechnol 35:438–440
Zong Y, Wang Y, Li C, Zhang R, Chen K, Ran Y, Qiu JL, Wang D, Gao C (2017b) Precise base editing in rice, wheat and maize with a Cas9–cytidine deaminase fusion. Nat Biotechnol 35:438–440

Chapter 6
Genomic Designing for Abiotic Stress Resistance in Jute

Jiban Mitra and Subhojit Datta

Abstract Jute (*Corchorus* spp.) being second to cotton in importance as fiber crop is grown on nearly 1.44 million hectares worldwide with production of 3.38 million tons jute fiber mainly in India, Bangladesh and as leafy vegetable to some extent in African countries. In addition, due to its importance as biodegradable source of different diversified product like biofuel, charcoal, paper, nanoparticle and its beneficial impact on protection of environment global demand of jute is increasing. Like other crops, this natural fiber crop encounters different abiotic stresses like drought, waterlogging, salinity, extreme temperature etc., which restrict the crop from expressing its full genetic potential resulting in reduction in fiber productivity and quality below optimum level. Plant responses to these abiotic stresses being both reversible and irreversible are dynamic and complex. Furthermore, as this crop is often exposed to a combination of stresses, responses of plant and adaptive mechanism become more complex and overlapping. In view of complexity of effect of different abiotic stresses as well as responses and adaptive mechanisms of plant to those, the progress on understanding the mechanism as well as evolving resistant genotype through conventional approaches has been limited in jute. Availability of genetic as well genomic resources of this crop along with novel innovative potential approaches towards enhancing abiotic stress resistance has been relooked for strengthening the future planning for further progress on it. The genomics approach integrating all possible ways of utilizing genetic and genomic resources and application of omics and reference genome information through systems biology is expected to result in genetic improvement in jute for abiotic stress resistance.

Keywords Abiotic stress · *Corchorus* · Drought · Genomics · Jute · Premature flowering · Resistance · Salinity · Waterlogging

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6.1 Introduction

Jute (*Corchorus* spp.), known as golden fiber, is of great importance as lignocellulosic bast fiber mainly for making sacks, hessian, carpet backing cloth, and value added diversified products (Kundu 1956; Kundu et al. 1959). Two cultivated species namely *C. olitorius* (tossa jute) and *C. capsularis* (white jute) are grown on an area of 1,437,939 hectares in the world in 2019 largely concentrated in Bangladesh contributing 51% and India contributing 46%; whereas in terms of production, India is the largest jute fiber producing country accounting for 51% followed by Bangladesh accounting for 47% of world production of 3,375,884 tons (FAOSTAT 2021). It has also been of paramount importance as leafy vegetable particularly in African countries due to its nutritional quality (Shanhua et al. 2010; Choudhary et al. 2013; Nyadanu et al. 2016; Ngomuo et al. 2017) along with medicinal value particularly as cardiac glycosides (Negm et al. 1980; Okoegwale and Olumese 2001; Khan et al. 2006; Kumari et al. 2018). Apart from socioeconomic contribution towards livelihood upliftment in large section of farm family, employment generation from jute agriculture, jute industry and trade and value added diversified jute product entrepreneurship, contribution and beneficial impact of this natural fiber crop towards environment is remarkable in terms of sustainable development. Jute, being a high CO_2-assimilating C_3 plant (Palit and Bhattacharyya 1984), absorbs 15 Mg ha^{-1} atmospheric CO_2, a major greenhouse gas, releases 11 Mg ha^{-1} O_2 in 110 days (Saha and Sagorika 2013) with sequestration of 4.8 Mg CO_2 per ton of jute fiber produced and moreover, improves the soil fertility through decomposition of falling leaves of 10–15 Mg ha^{-1} and of left-over root of 3 Mg ha^{-1} (Singh 2017). It indirectly helps in reducing deforestation as there are many products like jute composite for automobile, furniture, paper and pulp made from jute that substitutes the wood and therefore saves forest. Moreover, due to its biodegradable nature the application of jute fiber has been expanding in recent years as an alternative to plastic along with its use in paper industries (Das 1980), charcoal (Banerjee and Mathew 1985), renewable biofuel production (Singh et al. 2020) and nanotechnology for development of nano-cellulose, nano-lignin, and nano-carbon particle (Shah et al. 2021) resulting in increase in the global demand for jute.

On the other side of this positive impact and contribution of jute to protection of environment, this fiber crop experiences the different adverse effect of environment such as water stress (both deficit and excess), salinity, and extreme temperature throughout its life cycle and more specifically being rainfed and pre-kharif crop jute suffers from water-deficit stress during sowing and early growth stage in April as well as excess-water stress at later stage in June-July in addition to other abiotic stresses due to temperature, soil salinity etc. All these stresses restrict the full expression of genetic potential for plant growth, development, reproduction and ultimately reduce fiber productivity and quality below optimum level. Deciphering various adaptive mechanisms to cope up with abiotic stresses through different dynamic and complex plant responses to these, enhancing tolerance to drought and waterlogging with improved water-use efficiency and other stresses are nowadays becoming major

goal in breeding programs worldwide. But due to complex nature of the trait—resistance to abiotic stress with high genotype X environment interaction and lack of rapid efficient high throughput phenotyping/screening technique, breeding for resistance to different abiotic stresses has been very difficult with modest success. The availability of the reference genomes of both the cultivated species of jute (Zhang et al. 2021a, b, c) along with draft genomes (Islam et al. 2017; Sarkar et al. 2017) has laid a strong foundation to apply genomics-assisted breeding in jute. It is now possible to derive the precise information on genes conferring resistance, genotyping by sequencing of a diverse panel of genotypes with reference to different abiotic stresses. Large scale germplasm sequencing with assembly of pan-genome, directed genome editing and other innovative novel approaches will further aid the application of genomic information in jute improvement.

6.2 Abiotic Stresses in Jute

Like other crops, this natural fiber crop is not only exposed to various environment-induced stresses, a combination of different stresses instead of individual through its life cycle become limiting factor for growth and reproduction causing reduction in fiber productivity and deterioration of quality below optimum level. This also makes the response of crop to mitigate the effect of stresses through different morphological, physiological, biochemical, genetic adaptive mechanism more complex, overlapping and dynamic.

6.2.1 Water Deficit or Drought

Drought is the most important abiotic stress in jute particularly at early period during April due to scanty rainfall in jute growing areas of India and Bangladesh mainly affecting proper germination, seedling vigor, optimum plant stand, early plant growth and ultimately causing a serious threat to fiber productivity (Chaudhury and Basak 1969; Rao 1979; Prodhan et al. 2001a; b). Deep, profuse rooting architecture (Kar and De Sarkar 1961), well developed xylem element in stem (Kundu 1944), profuse mucilage in leave with moisture retention capacity make, in general, jute plant withstand the water deficit to some extent maintaining high osmotic value of about 11–15 atm during vegetative growth (Sengupta 1947) and *C. capsularis* is, more tolerant to water stress as compared to *C. olitorius* (Roy Chowdhury and Choudhuri 1986). But in case of severity in water-deficit stress particularly when seedlings are subjected to low humidity with soil moisture below 20%, the crop suffers from severe wilting and premature flowering (Patel and Mandal 1983) which hamper both fiber productivity and quality.

Plants develops various physiological, biochemical and genetic adaptations in combination to cope with or confer resistance to water deficit stress (Mitra 2001).

Drought escape, one of adaptive mechanisms through rapid phenological development and completion of life cycle or reproduction phage in advance of effect of water deficit is not beneficial for jute as drought occurs at early stages and at that time jute plant could not complete proper biogenesis of fiber which is the economic product in jute. However, developmental or phenotypic plasticity to recover the damage due to drought and regain plant vigor and growth when supplied with water is reported in jute (Fasinmirin and Olufayo 2009).

Other two mechanisms—drought avoidance based on maintenance of cellular plant water status through improvement of water balance by increased water uptake by deeper roots and/or reducing water loss through transpiration, and drought tolerance involving biochemical mechanisms like osmotic adjustment for dehydration tolerance, turgor maintenance by accumulation of osmo-protectants to enable plant to maintain functional growth under low available water are well-deciphered in jute. Unique and distinct root architecture of two cultivated species with deep tap root system in *C. olitorius* and shallow spreading root system in *C. capsularis* play important role in increased water uptake and in drought avoidance mechanism (Kar and De Sarkar 1961) and similarly increased water use efficiency and relative water content in leave, reduced stomatal conductance and transpiration rate in tolerant genotypes in *C. olitorius* in comparison to susceptible lines (Dhar et al. 2018) have been the key factor for drought avoidance mechanism.

As drought tolerance mechanism the presence of comparatively more soluble sugar in seed of *C. capsularis* (Bhaduri et al. 1985) decreases cellular water potential and thus, minimal hydration level for seed germination is attained faster than *C. olitorius* (Palit and Bhattacharyya 1981). Furthermore, accumulation of free proline (Palit and Singh 1991), increased level of hydrogen peroxide and lipid peroxide (Roy Chowdhury and Choudhuri 1985), increased total polyphenol (Dhar et al. 2018) in water deficit stress condition are also reported.

6.2.2 Anoxic Stress due to Waterlogging

Around 25% of total jute growing area being under low-lying land faces the waterlogging situation very frequently along with occurrence of intermittent submergence in medium land of jute-cultivated area under rainfed situation and this abiotic stress adversely affects the growth, yield and quality of jute fiber. Waterlogging reduces oxygen availability in soil pores as air is removed resulting in blocked gas exchange between soil and atmosphere and reduced oxygen diffusion rate in water-logged soil being 1/10000 of that in air. Consequently, hypoxia (restricted oxygen availability below optimum level) followed by anoxia (complete absence of oxygen) in long term occurs causing inhibition of root respiration and accumulation of toxic substances and this limits energy metabolism and restricts growth and developmental process, from seed germination to vegetative growth eventually leading to loss in fiber yield or even sometimes complete crop failure (Ghorai et al. 2003, 2005; Changdee et al. 2009). Severity of impact of waterlogging is found in the seedling stage in jute as young

seedlings even could not survive above field capacity and plant growth rate up to the age of six weeks is badly impaired by excess water (Wahab 1978; Prodhan et al. 2001a).

The major anatomic change in root as the key adaptive mechanism of plant in response to waterlogged condition is aerenchyma cell formation at the cortex of stem as observed in jute also (Changdee et al. 2008, 2009) along with formation of Casparian band and lignified cell wall in root cells, which prevents root rupture and oxygen loss from aerenchyma (Parvin et al. 2018). Aerenchyma being porous soft tissues with large intercellular spaces allows oxygen leak out into the roots and surrounding soil (Armstrong et al. 1991; Jackson and Armstrong 1999). In addition, adventitious roots formation at bottom of the stem close to water surface with horizontal growth (diageotropism) is another adaptive response to water submergence condition in jute (Parvin et al. 2018) having more access to oxygen than the original root system enabling gas diffuse between roots and shoots. However, *C. capsularis* is less sensitive to waterlogging stress due to shallow spreading root facilitating formation of more adventitious root for enhanced root respiration as compared to *C. olitorius* with deep tap rooting system. However adventitious root results in inferior quality fiber owing to hard barky bottom.

Plants also respond to waterlogging stress by altered energy metabolism and signalling cascades. Glycolysis and ethanol fermentation play important role in maintaining energy production to some extent during hypoxia caused by waterlogging. Waterlogging for long duration with anaerobic respiration ultimately causes accumulation of toxic metabolites with an increases in reactive oxygen species (ROS), notably hydrogen peroxide, thus eventually leading to cell death and plant senescence (Xu et al. 2014; Zhang et al. 2017a, b). Alteration of phytohormone metabolism either rapid accumulation or degradation is also caused as plant response to waterlogging (Hattori et al. 2009; Kuroha et al. 2018) along with the oxidative damage induced by the generation of ROS. Different types of antioxidants such as ascorbate peroxidase, superoxide dismutase, peroxidase, catalase, glutathione reductase, ascorbic acid, glutathione, tocopherols and carotenoids help in detoxifying the adverse effects of ROS. Ethylene plays an important role in adaptive mechanisms of plants in deficiency of oxygen inducing the activation of genes of enzymes associated with aerenchyma and adventitious root development, glycolysis and fermentation pathway (Pan et al. 2021).

6.2.3 Salinity or Salt Stress

Salinity or salt stress is a major factor restricting the expansion of jute cultivation in saline environments that would definitely reduce competition with food crops for arable land. Some jute growing area in southern coastal part of Bangladesh faces the adversity of salinity (Haque 2006) which has inhibitory effect on seed germination, seedling growth and vigor, plant stand of tossa jute with varietal difference in degree of resistance (Khandker et al. 1992; Khanom et al. 2018; Jui et al. 2021;

Mukul et al. 2021). In India also the effect of salinity in jute cultivation has been reported long ago (Basak 1951). However, *C. capsularis* is, in general, more tolerant to salinity than *C. olitorius* (Kar and De Sarkar 1957; Ghosh et al. 2013). Salinity-tolerant jute variety has great relevance where jute could be a promising crop in saline soils (Ma et al. 2009, 2011). In India a large coastal area of West Bengal could be explorable area for jute cultivation after mitigating salinity stress in those area and thus research on effect of salt and salinity and varietal difference both in *C. capsularis* and *C. olitorius* has been initiated (Sharma et al. 2012; Naik et al. 2015, 2019). Salinity stress in jute severely affects physiology and metabolism through creating osmotic stress, ionic imbalance and toxicity (Odunnaike et al. 2013) suppressing growth rates and biomass production. Primarily, seed germination is impeded due to osmotic stress-induced inhibition in imbibition affecting seedling establishment. Cellular metabolism is subsequently interfered as a result of ion toxicity and ROS-induced oxidative stress leading to reduction in photochemical efficiency and other physiological attributes in plants and ultimately fiber productivity (Ben-Yakoub et al. 2019).

Effects of salt stress like ion toxicity, oxidative stress damaging to cellular components—cell membrane, nucleic acids or lipids and metabolic dysfunction trigger a cascade of signal transduction pathways to change gene expression, energy metabolism, protein phosphorylation/dephosphorylation, and hormonal functional profile. Phytohormone abscisic acid (ABA) plays significant role in this signalling pathway with activation of mitogen-activated protein kinase pathways to phosphorylate many other ABA effector proteins. Plants also respond to ROS damage through modification in enzyme, protein and amino acid profile and production of carotenoids, phenolics such as phenolic acids and flavonoids and secondary metabolites as ROS-scavenging antioxidants (Abdallah et al. 2016).

In recent years, both at national and international levels, genomics and transcriptomics have been exploited to identify stress-tolerant genes regulated by salinity stress. The application of digital gene expression (DGE) analysis using next-generation sequencing strategy to transcriptomics study, allows in-depth characterization of transcriptional responses to stress. For the mining of novel alleles of stress tolerance in jute, genome-wide analyses were performed to identify aquaporins (*AQP*s), multifunctional stress protein family genes, in both tossa and white jute (Datta et al. 2018). Jute genome has been recently sequenced, and the first detailed genome-wide analysis of the *AQP* gene family in jute provides valuable information through functional analysis about their role in abiotic stress response.

6.2.4 Premature or Early Flowering

Early sowing of jute particularly during March results in pre-mature (early) flowering in *C. olitorius* and development of auxiliary branches at apex of main stem due to cessation of apical dominance with bushy, dwarf plants having little value for fiber both in terms of productivity and quality. More specifically, the early flowering of *C.*

olitorius would initiate after 30–45 days if sown in the month of March as compared to normal flower initiation time of 130–140 days if sown in middle of April. Photoperiodism is the major factor in causing this kind of stress. Being both the cultivated species short day plant with critical photoperiod requirement of 12.5 h, reproductive phase of jute is initiated under short photoperiod below 12.5 h whereas vegetative growth is favored by long photoperiod (Sengupta and Sen 1944, 1946). *C. olitorius* is, however, more sensitive to short light period for flowering than *C. capsuaris* (Sengupta and Sen 1952). In an attempt to understand other environmental factors controlling early flowering at early sowing in March through subjecting *olitorius* jute to all environmental conditions—varying soil water content, application of artificial rain, exposure of long day period of 14 to 16 h, it is observed that plants failed to initiate flower in any exposed condition when kept in either 14- or 16-h photoperiod revealing that the photoperiod has the main and largest effect on flowering (Sengupta and Sen 1947). However, apart from major contribution of photoperiodism, relatively small effect of low night temperature to enhance early flowering was reported in *C. capsularis* (Johansen et al. 1985), whereas jute under long day period is reported to have failed to initiate flowering irrespective of temperature up to 100 days and under short day higher temperature promotes early flowering at 45 days after sowing (Bose 1976).

But even when day length exceeds the critical level during vegetative growth stage, some instances of premature flowering in jute particularly in farmers' field have been recorded. This suggests that effect of environmental factors in addition to photoperidism on early flowering is yet to be identified distinctly. However, a delayed flowering mutant *(pfr 59)* induced in *C. olitorius* var. JRO 204 can effectively resist the early flowering under short day (Choudhury et al. 2019). Functional analysis of photoperiodic regulation of flowering-time trait in jute through comparative transcriptomics has identified ten sequences similar to known photoperiodic genes having possible functional role in regulating early flowering in jute. An epigenomic and transcriptomic study for deciphering major key biochemical pathways for flower development in jute is essentially required. Genome editing of candidate genes may be attempted to develop gene-edited jute lines with tolerance to premature flowering. However, some exotic lines from African origin such as Sudan Green from Sudan, Tanganyika 1 from Tanzania possess premature flowering resistance which have extensively been utilized for incorporating this trait into *olitorius* varieties.

6.2.5 Nitrogen Use Efficiency in Jute

Nutrition is an important aspect of jute production system particularly essential nutrients like nitrogen, phosphorus, and potassium. The supply and availability of these nutrients to plant at right quantity and time and with right method contribute significantly to its growth and yield (Karim et al. 1972). At the same time plant should also have potential and efficiency for uptake, utilization in terms of growth, photosynthetic and assimilate efficiency as source: sink ratio per unit of input and ultimately

to favour economic yield. In jute crop, nitrogen is the major element required for plant growth and has strong influence on fiber yield. Nitrogen deficiency reduces plant height and ultimately fiber yield. Nitrogen use efficiency (NUE) is the fraction of applied nitrogen that is absorbed and used by the plant for its growth and yield. NUE can be from agronomic point of view defined as the economic yield advantage gained per unit of N applied, absorbed, or utilized by the plant (Fageria and Baligar 2005). Genetic improvement in NUE in plant has great contribution to environmental sustainability.

ICAR-Central Research Institute for Jute and Allied Fibres (ICAR-CRIJAF), Barrackpore, India conducted preliminary experiments to screen germplasm and characterize their NUE using various morpho-physiological parameters. A number of jute genotypes were screened as potential NUE types based on the total biomass and enzymatic assessments (Mitra et al. 2016). In the following step, the detailed understanding of the mechanisms of N-uptake and utilization process, the genetic regulation of NUE trait, and identification of its quantitative trait loci (QTLs) in jute are considered as the priority for developing jute varieties efficient in N utilization.

The in-depth study of molecular basis of NUE and identifying N-responsive genes has been facilitated through genomics and transcriptomics with the advent of next generation sequencing (NGS) tools in large-scale analysis of genes expressing differentially under low and high N conditions to enable plants to use N more efficiently. Transcriptome sequencing revealed wide genotypic difference for nitrogen response and transcription factors played important role in driving genotype X nitrogen interactions at gene expression level in maize (Chen et al. 2015). Similarly, RNA-seq approaches also led to the identification of important N-starvation-responsive candidate genes from rice (Yang et al. 2016), sorghum (Gelli et al. 2014), and wheat (Curci et al. 2017). Further research on next generation sequencing (NGS) approach also led to the identification of single nucleotide polymorphism (SNP) markers and genetic map constructions, such as in spinach (Chan-Navarrete et al. 2016). Association mapping of NUE trait through a variety of markers like simple sequence repeat (SSR), kompetitive allele specific PCR (KASP), SNP markers has been worked out in rice (Liu et al. 2016), in barley (Han et al. 2016) and wheat (Guttieri et al. 2017).

6.2.6 Stress due to Heavy Metal in Soil

Heavy metals are a group of metallic elements that have relatively high densities, atomic mass (>23), and atomic numbers such as cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), zinc (Zn), copper (Cu), nickel (Ni), and chromium (Cr) and accumulate in soil due to various natural processes and anthropogenic (industrial) activities. Heavy metal—contaminated soil could bring alteration in morphological and physiological attributes in the crop plants leading to reduction in agricultural productivity. But jute has positive role in heavy metal related stress.

Recently, jute has been proved to have a great role in phyto-extraction of heavy metal toxicity from soil through the process of phytoremediation as it has ability to

germinate in metal-contaminated soil, absorb ionic compounds in the soil even at low concentrations through root system and accumulate considerable amounts of heavy metals being transported to the different above ground parts of the plants (Ahmed and Slima 2018; Saleem et al. 2020). Phytoremediation of heavy metals occurs in jute through root uptake, translocation, cellular compartmentation, and sequestration (Ogunkunle et al. 2015; Uddin et al. 2016; Abubakari et al. 2017; Saleem et al. 2019). Moreover, jute is of special importance as phytoremediation of heavy metals because of its use as fiber (not food) having no risk of bio-accumulation in human.

In a study of effect of Cu toxicity on the morphological and physiological changes in jute as well as of the potential and tolerance mechanism (Saleem et al. 2020) *C. capsularis* has been found to tolerate Cu concentrations of up to 300 mg kg^{-1} without significant decreases in growth or biomass. Furthermore, increasing levels of Cu in the soil caused oxidative damage in the leaves of jute plants which was overcome by the action of antioxidative enzymes. Copper is accumulated in the roots in earlier stages of the growth in higher concentration while transported to the above ground parts in the lateral stage of the growth. Similarly, relatively higher concentration of Pb, Cd, Cr, Cu, Fe, and Zn in the above-ground parts of the plants was observed as compared to roots of wastewater-irrigated plant. Hence, jute is a hyper-accumulator species for different heavy metals such as Pb, Cr, Cu, Fe, and Zn (Mazen 2004).

Exogenous application of salicylic acid and citric acid acting as chelating agent to increase bioavailabilty to plant roots enhances Cd, and Pb uptake (Hassan et al. 2016). Based on this information, exogenous application of organic acids or chelators is a useful strategy to reduce environmental risks associated with metal mobilization and an innovative approach for increasing metal accumulation by jute plants and biomass production.

6.2.7 Herbicide Application

Herbicide is, obviously, not as such any natural environment-induced stress but it has great relevance in crop plant like jute where weed infestation under hot and humid climate coupled with intermittent rainfall during the jute-growing season results in severe jute-weed competition and ultimate yield loss up to 70% (Ghorai et al. 2006) and moreover weed management through chemical and manual weeding enormously increases expenditure accounting for 30% of total cost of jute cultivation (Saraswat 1999) and also adds to environmental pollution. Although herbicide is used to control only unwanted weed plants naturally grown along with crops through inhibiting their growth in order to enable crop plants become free from completion for nutrients, water, light, sometimes the use of herbicide has unintended consequence, when applied inappropriately, of injuring non-target crop plants implying the need for judicious and eco-friendly weed management to reduce such herbicide-induced stress on crop plants. In view of severe competition from *Cyperus rotundus* and broad leaved weeds faced by jute crop (Kumar et al. 2013), non-availability of selective herbicides to control broad leaved weeds and sedges in jute and high cost of manual weeding,

judicious application of broad spectrum herbicide having potential to stabilize the fiber productivity as well as to bridge the fiber yield gap at farmers' field (Kumar et al. 2017) will be an option only if crop plants are made herbicide-resistant and the first report of herbicide-resistant weed in the early 1970s (Ryan 1970) triggered interest in mimicking this unintentional development for use in crop breeding. But at the same time the issue of environmental hazards due to herbicide application remains unsolved.

Genetic engineering involving strategies of isolation and introduction of a gene from another organism, mostly bacteria, which is able to overcome the herbicide-induced metabolic blockage has been applied for developing herbicide resistant transgenic crops. Moreover, herbicide tolerance is the most widely adopted (~75%) transgenic crop trait. Herbicide resistant crops have been grown commercially since 1984, when the first triazine-resistant rapeseed (*Brassica napus*) cultivar (OAC Triton) developed at University of Guelph was marketed in Canada. In jute (*C. capsularis* var. JRC 321), an in vitro plant regeneration and genetic transformation protocol through particle bombardment method was established using one-day-old apical meristematic tissues of germinating seedlings as explants and bialaphos (herbicide) resistance gene (*bar*), synthetically designed for high level plant expression (Bhattacharyya et al. 2015). However, this technology is marred with regulatory labyrinth, randomness of gene integration site, copy number, cost and time issues.

On the other hand, genome editing technology enabling modifications of DNA sequences with great precision and perfection offers great promise for crop improvement. CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated 9 protein) has revolutionized genome editing due to simplicity and versatility. The editing technology has been successfully applied for developing herbicide tolerant rice and maize providing the proof of principle of this powerful tool. During the past decade, several genome editing technologies, zinc fnger nucleases (ZFNs), mega nucleases, transcription activator-like effector nucleases (TALENs), and recently, cas9 protein and guide RNA (Cas9-gRNA) systems, were developed allowing modification of a gene product or metabolic pathway in a desired way. Application of the Cas9-gRNA technology for editing acetolactate synthase (*ALS2*) gene resulting in chlorsulfuron resistance in maize has been successful (Svitashev et al. 2015). Herbicide-resistant rice lines have been developed through CRISPR/CAS9-mediated editing of *ALS* by Sun et al. (2016). They designed a strategy using two gRNAs simultaneously and suggested that successful strategy in one plant species needs to be modified for other plant species. Highly tolerant herbicide-resistant transgenic rice was developed by Chhappekar et al. (2015) by expressing codon modified synthetic 5-enolpyruvul shikimate-3-phosphate synthase (*EPSPS*) which could tolerate up to 1% commercial glyphosate. International Centre for Genetic Engineering and Biotechnology, New Delhi is working on developing transgenic rice resistant to several non-selective herbicides at a time with different mode of action (Fartyal et al. 2018).

At ICAR-CRIJAF, attempts are being made to develop an efficient method to introduce multiple discrete point mutations in the jute *EPSPS* and *ALS* gene using CRISPR/Cas9 to make major herbicides glyphosate and imazethapyr non-toxic to

jute plant. The available jute transcriptomes were searched for *EPSPS* gene and transcripts were identifiedl. Nucleotide and protein BLAST analyses revealed that these share considerable homology and share conserved motifs with other higher plant *EPSPS* gene. Jute *EPSPS* was found to share maximum homology with cacao (*Theobroma cacao*) followed by *Gossypium raimondii* and upland cotton. Using bioinformatics tools, glyphosate-*EPSPS* interaction sites were predicted and key amino acids were identified. Amino acids playing a major role in glyphosate binding were identified which are potential targets for mutation and genome editing (Datta 2018).

6.3 Genetic Resources and Conventional Breeding

Plant genetic resources are of great importance in genetic improvement in any crop plant through conventional or innovative breeding, marker-based breeding and also in creation of genomics resources in the form QTL analysis, omics analysis, allele mining, whole genome sequencing including reference genome. In this regard, jute is rich in species diversity including wild relatives, germplasm accessions, obsolete and novel varieties.

6.3.1 Wild Species and Wild Relatives of Corchorus

Out of over 170 *Corchorus* species in the Malvaceae (more accurately Sparrmanniaceae) family as reported in 'Index Kewensis', *C. capsularis* (white jute) and *C. olitorius* (tossa jute) domesticated mainly for fiber are the commercially important cultivated species. The others being wild in nature, are mostly distributed in the tropical/subtropical regions of Africa, America (including Brazil, Mexico, Bolivia, Venezuela, and West Indies), Australia, China, Taiwan, India, Myanmar, Bangladesh, Nepal, Sri Lanka, Japan, Indonesia, Thailand, Malaysia, and Philippines (Wild 1984; Brands 1989–2021; Edmonds 1990; Heywood 1993). The primary center of origin of *C. olitorius* and diversity of the wild taxa of *Corchorus* appears to be Africa, where large numbers (nearly 46) of species have been reported including cultivated species *C. capsularis* and *C. olitorius* (Kundu 1951; Aluka 2006–2021: http://www.aluka.org) mainly concentrated in eastern and southern part of the continent. South Africa is the richest source of diversity with 16 species followed by Tanzania with 13 species and Zimbabwe, Ethiopia, Mozambique each with 12 species and Kenya with 11 species. Zambia has nine species of the wild taxa of *Corchorus* (Edmonds 1990).

In Asia, wild species are distributed in India, Bangladesh, Pakistan, Thailand, Indonesia, and *C. capsularis* is assumed to be native of Indian subcontinent and specifically Indo–Myanmar including South China has been considered as center of origin of *C. capsularis* due to omnipresence of this species in this region although

there are still controversies (Kundu et al. 2013). In addition to two cultivated species, eight wild species of *Corchorus* (*C. aestuans, C. depressus, C. fascicularis, C. pseudo-olitorius, C. tridens, C. trilocularis, C. urticifolius,* and *C. velutinus*) have been reported from India and most being migrated from Africa (Mahapatra et al. 1998). *C. aestuans* is the most dominating wild species of India distributed all over area followed by *C tridens* and *C. trilocularis* restricted to western, central and southern part of the country. *C urticifolius* and *C. pseudo-capsularis* are distributed in Tamil Nadu, while *C. pseudoolitorius* in the western part, *C. depressus* in the semi-arid region of Gujarat, Rajasthan, Tamil Nadu, and Punjab (Mathur and Sundaramoorthy 2008).

Most of the wild species of *Corchorus* being shy in fiber biogenesis are potent sources of biotic and abiotic stress tolerance coupled with finest quality of fiber (Palve et al. 2004; Mahapatra and Saha 2008). *C. depressus, C. asplenifolius, C. cinerascens, C. erinoceus,* and *C. erodiodes* are highly drought tolerant whereas *C. tridens* and *C. trilocularis* show significant level of tolerance to water stagnation. *C. siliquosus*, *C. hirtus*, and *C. sulcatus* are highly adaptable in shallow soil.

6.3.2 Plant Genetic Resources Management

The International Jute Organization (IJO), Dhaka, Bangladesh established in 1984 initiated germplasm exploration missions in species diversity rich ecological niche of Tanzania and Kenya under leadership of Dr. J. M. Edmonds, a herbarium consultant and a technical report on distribution pattern of *Corchorus* in Africa was published (Edmonds 1990) depicting the germplasm potential of 12 different *Corchorus* wild species—*C. aestuans, C. baldaccii, C. brevicornutus, C. fascicularis, C. olitorius, C. pseudo-capsularis, C. pseudoolitorius, C. shimperi, C. tridens, C. trilocularis, C. urticifolius*, and one unknown species collected. Later explorations were also made in different countries including China, Indonesia, Nepal, Thailand, and Pakistan to collect wild *Corchorus* germplasm from their natural habitats along with acquisition through correspondence with CSIRO (Australia), USDA (USA), CENARGEN (Brazil), and IBPGR (Italy) (Arangzeb 1988). As a result, a total of 2,300 accessions were collected by IJO and distributed to different countries for evaluation, conservation, and utilization in *Corchorus* breeding program. The Gene bank of the Germplasm Division, Bangladesh Jute Research Institute (BJRI) is functioning as the IJO Centralized Germplasm Repository (CGR) and at present a total of 4,081 accessions of plant genetic resources comprising 15 species of *Corchorus* are under conservation at −20 °C as base collection. In India, till early 1970s, plant breeders had worked with a gene pool of mere 300 accessions, mainly of local collections, of both cultivated and eight wild relatives of *Corchorus*. Systematic PGR management activities were initiated in 1977 through direct explorations and correspondence (Mahapatra et al. 1998). Majority of the collections in India were made by the ICAR-CRIJAF and ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) of the Indian Council of Agricultural Research (ICAR). Later on, during 1999–2004

under the National Agricultural Technology Project (NATP) 655 accessions covering landraces and wild relatives of *Corchorus* were collected from different agro-climatic regions and characterized. At present ICAR-CRIJAF have active collection of 3406 *Corchorus* accessions comprising 968 *C. capsularis*, 1827 *C. olitorius*, and 611 wild jute representing eight species being conserved in the mid-term gene bank of ICAR-CRIJAF and in the National Gene Bank at ICAR-NBPGR, New Delhi as base collections (Mahapatra et al. 2006; Karmakar et al. 2014).

6.3.3 Genetic Diversity

Estimation of genetic diversity of 192 *C. olitorius* and 216 *C. capsularis* accessions of IJO, inclusive of certain induced mutants of Indian cultivars (Palit et al. 1996) revealed high diversity in characters such as plant height, harvest index, cambial activity and fiber strength within each origin country and mutants. Irrespective of specific origins and traits, cambial activity (measured as fiber: wood ratio) showed lowest and plant height showed highest diversity. Kumar et al. (2008) characterized all released or notified varieties including common knowledge varieties of both species using 17 morphological characters for distinctness, uniformity and stability (DUS) testing. Out of 17 morphological traits, two were polymorphic, seven dimorphic and eight monomorphic, in *C. capsularis*. In contrast, *C. olitorius* was represented by nine polymorphic and eight dimorphic traits. In addition, several reports on genetic diversity on different panel of germplasm for different quantitative traits mainly fiber related have been reported with varied degree of diversity (Palve and Sinha 2005; Yumnam et al. 2016; Jatothu et al. 2018; Mukul et al. 2020a, b).

Genetic diversity at molecular level having advantage over phenotype due to absence of environment effect unlike morphological traits of various jute varieties and accessions collected from diverse locations, using different molecular marker like random amplified polymorphic DNA (RAPD) (Qi et al. 2003a; Haque et al. 2007), inter-simple sequence repeat (ISSR) (Qi et al. 2003b), SSR (Akter et al. 2008; Mir et al. 2008a, b), insertion-deletion (InDel) (Zhang et al. 2017a, b; Yang et al. 2018), RAPD and amplified fragment length polymorphism (AFLP) (Hossain et al. 2002, 2003), AFLP and SSR (Basu et al. 2004), sequence tagged microsatellite (STMS), ISSR and RAPD (Qi et al. 2004; Roy et al. 2006) have been reported and it is revealed that although inter-specific polymorphism is highly prevalent, intra-specific polymorphism is comparatively low particularly among varieties.

6.3.4 Conventional Breeding

The genetic improvement of jute through conventional breeding has so far been confined to intraspecific hybridization due to failure in interspecific hybridization in effective way. Several attempts on interspecific hybridization between the two

cultivated species of *Corchorus* (*C. capsularis and C. olitorius*), and simultaneously between the cultivated and wild species were made over the last five decades by many workers, but its successful utilization in breeding is lacking (Swaminathan and Iyer 1961; Islam and Haque 1967; Mia and Shaikh 1967; Islam and Rashid 1960; Islam 1964; Arangzeb and Khatun 1980; Sinha et al. 2004; Maity and Datta 2008). However recently at ICAR-CRIJAF successful hybridization between *C. Capsularis* and *C. olitorius* using *capsularis* as maternal parent and stabilization of its advanced generation at F_8 with 194 lines (Sarkar 2019) with creation of prebreeding genetic resource with large genetic variability have been reported. Furthermore, *C. aestuans* has also been utilized as useful donor in breeding program (Kumar et al. 2019).

The traditional breeding method has contributed significantly towards premature flowering resistance. Premature flowering at early sowing during first fortnight of March makes *olitorius* jute unsuitable for jute-paddy cropping sequence. Identification of premature flowering resistance in an exotic African germplasm accession-'Sudan Green' and development of three varieties JRO 878 (JRO 620 X Sudan Green), JRO 8735 (JRO 632 X Sudan Green) and JRO 524 (Sudan Green X JRO 632) (Chakraborty and Ghosh 1969) with premature flowering resistance introgressed from this accession through pedigree method during seventies and two more varieties JRO 8432 (IC 15,901 X Tanganyika 1) in 1999 (Chowdhury and Das 2003) and JRO 128 (TJ 6 X Tanganyika 1) in 2002 using another premature flowering resistant accession 'Tangayika 1' (Joseph and Saha 1978)) changed the scenario of cultivation of jute towards predominance of cultivation of *olitorius* in 90% of total area under jute. However, development of such premature flowering resistant *olitorius* varieties with higher adaptability, fiber productivity and fiber quality namely S 19, Tarun, JRO 204, JBO 2003 H, CO 58, JBO 1, JRO 2407 has been continuing (Kar et al. 2010; Pandey et al. 2020).

For breeding for resistance to other stresses, screening of different germplasm accessions including varieties has been accomplished. Identification of accession through screening based on tolerance index for root characters to be used as donor parent in hybridization program for resistance to drought in *olitorius* jute (Sawarkar et al. 2016), screening germplasm for salt tolerance (Ma et al. 2009; Ghosh et al. 2013), salinity tolerance in both the cultivated species based on physiological traits (Ma et al. 2011; Islam et al. 2011; Naik et al. 2015; Taneenah et al. 2015) have been reported.

Resistance to abiotic stress is a polygenic trait involving the expression of many sets of genes in different biological pathways with low heritability with higher level of genotype x environment interaction in its phenome. Plant response to the abiotic stress with well-organized system of sensing the environmental signals comprises a set of events including stress signalling, stress transduction and gene expression through biosynthesis and bio-action of transcription factors, stress-related proteins, enzymes and metabolites. Due to this complexity in expression of abiotic stress resistance, conventional breeding being tedious and time-consuming approach is less effective with limited success in bringing genetic improvement of resistance to abiotic stresses and moreover lack of efficient, high through-put screening techniques for different abiotic stresses is the added constraint.

In this respect, genomic approach including all 'omics' (genomics, transcriptomics, proteomics, metabolomics) for identifying stress-related transtriptomes, proteomes, metabolomes and structural and functional dimension of relevant genes and whole genome sequencing including reference genome for in-depth deciphering abiotic stress responses at the molecular level would be appropriate in bringing success in abiotic stress resistance through molecular breeding (marker-assisted selection/breeding, transgenic and genome editing).

6.4 Genomic Resources Available for Jute Improvement

In view of ineffectiveness of conventional breeding to bring genetic improvement in abiotic stress resistance and application of genomics approach as way forward to lead to success in this endeavor, availability of genomic resources available in jute has great relevance and importance to proceed ahead.

6.4.1 Linkage Maps and QTL

High-density genetic map mainly based on DNA-based markers being foundation for identifying QTLs have been available in both *C. olitorius* and *C. capsularis* in last few years and a number of QTLs recently identified on the basis of those linkage maps would serve as genomic resources for genomics-aided breeding in jute.

The first and preliminarily linkage map with three linkage groups covering 87.3 cM with an average marker interval of 8.73 cM using ISSR marker in an F_2 population from a cross between two *C. olitorius* jute genotypes differing in cold tolerance was constructed in 2006 (Sultana et al. 2006) and subsequently in 2008 another linkage map based on RAPD marker (Haque et al. 2008) was developed. Chen et al. (2011) developed a genetic linkage map using sequence-related amplified polymorphism (SRAP) marker with an average marker interval of 17.86 cM.

Construction of a comprehensive SNP-based linkage map for the first time in *C capsularis* from a F_6 RIL population derived from cross between two genotypes contrasting for stem rot resistance covering cumulative length of 2016 cM with marker interval of 4.2 cM (Biswas et al. 2015) would be a useful genomic resources.

In *C. olitorius* jute through QTL analysis using a mapping population derived from singe seed descent method of a cross JRO 524 (high yielder, coarse fiber) and PPO 4 (fine fiber) and SSR-based linkage map (developed by Das et al. 2012a) 21 QTLs for eight fiber yield traits and only one QTL for fiber fineness have been identified for the first time through single locus analysis (Das et al. 2012b) and 11 M-QTLs have also been identified for seven fiber yield traits (excluding top diameter) and one M-QTL for fiber fineness using two-locus analysis involving QTL Network. For six fiber yield traits, 16 E-QTLs involved in nine QQ epistatic interactions and for fiber fineness, four E-QTLs involved in two QQ epistatic interactions and for

fiber strength, six E-QTLs involved in three QQ epistatic interactions have also been identified. Eight M-QTLs observed for the fiber yield traits are also involved in QE interactions; for fiber fineness and fiber strength, no QE interactions are observed.

Further using an F_6 RIL population in *C. olitorius* and SSR-based linkage map on the seven linkage groups covering genetic distance of 799.9 cM, through single marker analysis in combination with MQM mapping 26 definitive QTLs have been detected for bast fiber quality, yield and yield related traits distributed on six linkage groups (Topdar et al. 2013).

Using an F_3 population in *C. olitorius* from a cross between Sudan Green and bfs (bast fiber shy) mutant and RAD (restriction-site-associated DNA) markers based linkage map in seven linkage groups spanned 358.5 cM, nine QTLs across the two environments have been identified. The QTLs for fiber content was coincident with one QTL each for fiber yield, plant height, root weight, basal diameter on top of a single-SNP (C/T) marker at 40.2 cM on linkage group 1, each accounting for 7–11% of the phenotypic variance. Two QTLs linked in repulsion one each for plant height and basal diameter, with varying degrees of overdominance, were associated with two single-SNP (C/T) markers on linkage group 2, each accounting for 17–18% of the phenotypic variance (Kundu et al. 2015).

From a linkage map of 913 SLAFs specific locus amplified fragment sequencing (SLAF-seq) markers using F_8 population of *C. capsularis*, 11 QTLs for plant height including one major effect QTL across two cultivation locations, with each QTL accounting for 4.14–15.63% of the phenotypic variance have been identified (Tao et al. 2017).

From a high-density genetic map with 4839 SNP markers spanning 1375.41 cM and an average distance of 0.28 cM between adjacent markers on seven linkage groups using an F_2 jute population, three obvious and 13 minor QTLs involved in salt tolerance have been identified for the first time on four linkage groups explaining 0.58–19.61% of the phenotypic variance. The major QTL, *qJST-1*, has been detected under two salt stress conditions that explained 11.81 and 19.61% of the phenotypic variation, respectively (Yang et al. 2019).

6.4.2 Transcriptomics

Transcriptomics being convenient rapid technology for assessing gene expression as well as regulation to elucidate the molecular mechanism underlying the development and biogenesis of different tissues and organ in term of biosynthetic pathways utilizes next generation high-throughput sequencing technology to sequence cDNA, and it can comprehensively acquire information for all transcripts under a certain condition of growth, tissues or organ. The expression profiles of a large number of genes responsible for stress-related effector and regulatory proteins are altered in response to different abiotic stresses in plant. Comprehensive transcriptomic analysis will effectively elucidate gene function of differentially expressed genes (DEGs) under different stresses and explore the molecular mechanism of abiotic stress resistance.

A putative leucine-rich repeat receptor-like protein kinase (*LRR-RLK*) gene together with its 5′ and 3′ untranslated regions in tossa jute has been identified to play role in abiotic stress-response signal transduction pathway as low temperature, dehydration, high salt concentration, abscisic acid are found to induce expression of this gene (Alam et al. 2010). Similarly, another novel dehydration responsive transcript *drp* has been identified from tossa jute and found to be expressed in tissues of root, stem and leaf with decreased expression under dehydration stress (Sharmin et al. 2011).

A comprehensive transcriptome analysis for leaves, stem bast, flower, fruit of *C. olitorius* at different growth stage detected 77% of known gene 28540 in reference genome and 4772 novel genes with specified up-regulated and down-regulated genes in those tissues. Biosynthesis pathway of secondary metabolites enriched in stem with 26 up-regulated genes, phenylpropanoid and flavonoid with 53 genes enriched in flower were discovered (Yang et al. 2020b).

Whole transcriptome sequencing of jute fiber cell (Ahmed et al. 2020) identified a total of 66 fiber-related genes where nine were involved in fiber cell initiation and elongation process and remaining in secondary cell wall development and gene ontology analysis revealed 14,144 genes (52.21%) to be involved for biological process, 8399 (31%) for molecular function and 4549 (16.79%) for cellular components.

Among the two cultivated species of jute, *C. olitorius* is considered as drought tolerant *C. capsularis* as drought sensitive species. A comparative transcriptome analyses between the two species identified higher number of DEGs in drought induced genotype of sensitive *C. capsularis* (794) as compared to that of tolerant *C. olitorius* (39) (Yang et al. 2017). This is ascribed to the fact that as compared to tolerant species, sensitive species undergo greater changes in phenotype, physiological and biochemical properties when mitigating the effects of stress conditions. Of those, 567 and 7 DEGs were up-regulated and 227 and 32 were down-regulated in *C. capsularis* and *C. olitorius*, respectively with only 7 common DEGs, all being down-regulated. Gene ontology annotation in *C. olitorius* showed DEG enrichment only in catalytic activity having role in adaptation to drought stress through modifying physio-biochemical process. In *C. capsularis*, under drought stress, peroxisome and phenylpropanoid biosynthesis pathway have been found to be involved in scavenging of ROS, where 14 unigenes have significant differential expression in response to increase of superoxide.

Transcription factor *NAC* [(no apical meristem (*NAM*), Arabidopsis transcription activation factor (*ATAF*), and cup-shaped cotyledon (*CUC*)] are found to play important roles in abiotic stress in addition to regulation of developmental processes. Such a *NAC* factor in *C. capsularis*- *C. capsularis* NAC1 (*CcNAC1*) was found to express under drought stress (20% PEG) to mitigate the negative effect by speeding the early maturity of *C. capsularis* (Zhang et al. 2021a). Additionally, Zhang et al. (2021b) identified a new gene- no apical meristem-2-like (*NAM-2-like*) genes (a member of the *NAC* transcription factor family) having significant regulatory role in influencing 3-ketoacyl-CoA synthase (*KCS*) gene which is responsible for wax biosynthesis whereas accumulation in cuticular wax confer drought resistance of

drought tolerance in *C. capsularis*. Over-expression of *NAM-2-like* gene resulted in up-regulation of *KCS* gene and increased drought tolerance and RNAi knockdown lines of this gene became drought sensitive. Similarly a gene- *C. capsularis* NAC1 (*CcNAC1*) gene, one of *NAC (NAM, ATAF1* (Arabidopsis transcription activation factor 1), *ATAF2,* and *CUC2* (cupshaped cotyledon 2) transcription factor (Zhang et al. 2020) regulating *KCS* have been found to be responsible for drought resistance.

Transcriptome analysis of jute from diverse tissues of leaves, roots, stem bast and stem stick at a vigorous vegetative growth stage has identified a broad range of unigenes (48,914 unigenes with an average length of 903 bp) associated with vegetative growth which has further resulted in development of large-scale SNPs (12,518). Gene expression analysis of identified gene involved in cellulose biosynthesis for bast fiber namely *5 Susy, 3 UGPase, 9 CesA, 18 CSL, 2 KOR*, and *12 COBRA unigenes* has provided understanding of the mechanism of fiber development in jute (Zhang et al. 2015).

Comparative transcriptome analysis and weighted gene co-expression network analysis for different tissues of *C. capsularis* at different stages (mature flowers, leaf tissues of vegetative growth period and of flowering period, fruits of diameter of <0.8 cm and of diameter of >0.8 cm, bast of vegetative growth period and of flowering period) have identified various tissue-specific DEGs and 12 co-expression modules comprising 126 to 4,203 genes, associated with the development of various tissues (Yang et al. 2020a). Further a comprehensive database of gene expression of various tissue development with a network of 21 genes regulated by transcription factor OMO55970.1, some being involved in the development of stem bast and fiber has been reported.

Bast transcriptomes of a deficient lignified phloem fiber (*dlpf*) mutant and its wild-type jute cv. JRC 212 (*C. capsularis*) (Chakraborty et al. 2015) identified 16 and 10 genes of the upstream shikimate-aromatic amino acid and downstream monolignol biosynthetic pathways, respectively. Phenylalanine ammonia-lyase 1 (*CcPAL1*) was found to be co-down-regulated with several genes of the upstream shikimate pathway in mutant bast tissues at an early growth stage, although its expression relapsed to the normal level at the later growth stage. Whereas cinnamyl alcohol dehydrogenase 7 (*CcCAD7*) was strongly down-regulated in mutant bast tissues irrespective of growth stages and accompanied by co-upregulation of SCW (secondary cell wall)-specific genes cellulose synthase A7 (*CcCesA7*) and fasciclin-like arabinogalactan 6 (*CcFLA6*) at an early growth stage, which was predicted to be involved in coordinating the S-layers' deposition in the xylan-type jute fibers. Lignin biosynthesis occurs in jute fibers via well-conserved shikimate-AAA and monolignol pathways and CAD would be a promising target for developing low-lignin jute fibers using genomics-assisted molecular approaches.

For identification of photoperiod-responsive transcripts with their role in regulating flowering mechanism in jute under short-day conditions transcriptome analysis from shoot apex tissues of a delayed-flowering mutant (*pfr59*) and its wild-type cultivar JRO 204 grown under short-day conditions was undertaken (Choudhary et al. 2019) and 240 differentially expressed transcripts (DETs) showing homology

to 75 and 42.5% sequences from the NCBI-NR and SWISSPROT databases, respectively have been identified. Gene expression validations of nine putative photoperiod-related DETs showed their differential expression patterns in leaf and shoot apex tissues of *pfr59* and JRO 204 under short-day conditions. Homology-based search approach of 240 DETs from *pfr59* and JRO 204 against the FLOR-ID genes related to photoperiodism database identified 10 DETs having significant differential expression in the mutant compared to JRO 204 showed significant match to FLOR-ID proteins. The gene *Co-KSB*, coding for ent-kaurene synthase B enzyme showed most striking changes in their expression profiles in both leaves and shoot apex of *pfr 59* and JRO 204 and this enzyme is involved in the gibberellin (GA) biosynthetic pathway and thus, have a crucial and a positive effect on flowering-time along with three transcripts, coding for Gibberellin 20 oxidase 1 (*GAOX1*), Gibberellin receptor GID1B (*GID1B*), and Gibberellin receptor GID1C (*GID1C*), having significant changes in gene expression between *pfr 59* and JRO 204. GA-dependent pathway is one of the major flowering-time regulation pathways known to play important role in the transition from vegetative to flowering stage in model *Arabidopsis.*

Identification of 2079 high-quality eSSR markers and integrating them in a JuteMarker database for wider accessibility is one of the first efforts in bioinformatics in jute and allied fibers (Saha et al. 2017).

6.4.3 Genome Sequencing

The 2C DNA content in *C. capsularis* cv. D 154, *C. olitorius* cv. O 4 and their F_1 hybrid was first estimated to be 2.3, 2.8 and 3.1 pg, respectively (Samad et al. 1992). Based on this genome size (1C DNA amount) was also estimated 1100 and 1350 Mb for *C. capsularis* and *C. olitorius*, respectively (Mir et al. 2009). Later on nuclear DNA content (2C values) being 0.502–0.695 pg for *C. capsularis*, and 0.643–0.718 pg for *C. olitorius* and haploid genome (1C) sizes of ~280 Mb and ~324 Mb of *C. capsularis* and *C. olitorius*, respectively, was accurately estimated by Sarkar et al. (2011) through flow cytometric analysis. *C. fascicularis* was reported to have the smallest haploid genome (~188 Mb) followed by *C. aestuans* (~194 Mb). On an average, genome sizes (1C values) are about one-fourth of their corresponding earlier-reported estimates. JRC 212 variety of *C. capsularis* has the smallest genome (~246 Mb) among the cultivated species.

Islam et al. (2017) reported high quality draft genomes of both cultivated species covering 91.6% and 82.2% of the estimated genome sizes for *C. olitorius* (448 Mb) and *C. capsularis* (405 Mb), respectively with identification of a total of 37,031 (*C. olitorius*) and 30,096 (*C. capsularis*) genes validated by cDNA and RNA-seq data and their comparisons at the functional genomics level to support tailor-designed breeding. This work has improved understanding of the molecular basis of fiber biogenesis.

The draft genome (377.3 Mb) of *C. olitorious* cv. JRO 524 (Navin), a leading jute variety has been sequenced (Sarkar et al. 2017) and a total of 57,087 protein-coding genes including a large number of 1765 disease resistance-like and defense response genes have been identified. Seven chromosome-scale genetically anchored pseudomolecules were constructed to use synteny analysis with the cocoa (*Theobroma cacao*) and cotton (*Gossypium raimondii*) genomes as the highest sequence similarities in annotated genes with cocoa followed by cotton was observed. A total of 185,698 genomic SSRs, with mononucleotide repeats being the most abundant class (76.0%), followed by di- (16.0%), tri- (5.7%), tetra- (0.8%), penta- (0.2%) and hexa-nucleotide (0.2%) repeats have been identified. (Sarkar et al. 2017).

Furthermore, very recent reference genome of both *C. olitorius* (genome of 361 Mb with 28,479 genes) and *C. capsularis* (genome of 336 Mb with 25,874 genes) based on population structure analysis of 57 varieties of *C. olitorius* and 242 *C. capsularis* by whole genome sequencing, identification of candidate genes for fiber biogenesis and quality along with marker-trait association for fiber fineness, cellulose content, lignin content (Zhang et al. 2021a, b, c) has been of paramount importance. These resources are expected to be used in deciphering adaptive mechanism of different abiotic stresses through translational genomics as well in identification of abiotic stress related candidate genes to unfurl the tolerance mechanism. However, using this reference genome very recently a total of 160,173 and 154,715 SSRs of *C. olitorius* and *C. capsularis,* respectively have been identified along with the first physical map in *C. capsularis* with 535 polymorphic SSRs (Niyitanga et al. 2021).

All these genomic resources being the focus of future research would help to elucidate the molecular adaptive mechanism for abiotic stress resistance to promote molecular breeding.

6.5 Modern Biotechnological Approach

6.5.1 Transgenics in Jute

To overcome the sexual incompatibility barrier during transfer of suitable genes into elite background, plant genetic transformation has been the most potent approach. For successful application of genetic transformation technology in crop improvement, it is imperative to have a robust regeneration system. However, *Corchorus* species and major jute cultivars are recalcitrant to in vitro regeneration and genetic manipulation. In several instances results are found to be highly dependent on genotypes in *C. capsularis* (Naher et al. 2003) and *C. olitorius* (Khatun et al. 2003). Preformed regenerable explants were found to improve the results of regeneration to some extent (Ahmed et al. 1989; Seraj et al. 1992; Hossain et al. 1998; Islam et al. 1999; Sarker et al. 2008; Bharadwaj et al. 2011; Amin et al. 2012). *Agrobacterium*-mediated genetic transformation with marker genes have been carried out in

many jute varieties (Hossain et al. 1998; Bharadwaj et al. 2011; Saha et al. 2014). Bhattacharyya et al. (2015) established an efficient and reproducible in vitro plant regeneration and genetic transformation system in jute using particle-gun bombardment approach with bialaphos resistance gene (bar) as marker. However, Islam et al. (2013) have shown that transgenic *C. olitorius* jute plants expressing bacterial *katE* gene exhibited oxidative stress tolerance under salinity via upregulation of ROS scavenging enzymes.

Latest in 2018, *C. capsularis* var. JRC 321 have been transformed with *bar* gene via *Agrobacterium* transformation and analyzed up to T_2 generation. Stable integration and expression of transgen in the jute genome was confirmed by growing transgenic plants in glufosinate ammonium containing media and by spraying glufosinate herbicide (Basta) (Majumder et al. 2018).

Under the ICAR-Network Project on Transgenics in Crops (ICAR-NPTC) funded project on "Herbicide Tolerance in Jute" ICAR-CRIJAF has made significant progress on studying herbicide tolerance and in vitro culture system in *C. olitorius* jute. Jute germplasm have been screened for tolerance to glyphosate both in vitro and in field, standardized *in-vitro* shoot regeneration by implementing different regeneration systems in jute using explants like cotyledonary petioles and shoot tips. JRO 2407 and JRO 524 showed better regeneration compared to JRO 632. Shoot apical meristem explants were standardized as the explant for genetic transformation (*Agrobacterium tumefaciens* mediated) in tossa jute (Datta 2016).

6.5.2 Genome Editing in Jute

The emergence of sequence-specific nucleases that enable genome editing is revolutionizing basic and applied biology. Since the introduction of CRISPR–Cas9, genome editing has become widely used in transformable plants for characterizing gene function and improving traits, mainly by inducing mutations through non-homologous end joining of double-stranded breaks generated by CRISPR–Cas9. Plant transformation is a major bottleneck for realizing the potential of plant genome editing and it would be highly desirable to perform precision gene editing in plants, especially in transformation-recalcitrant species like jute.

Other than agricultural applications, CRISPR system is expected to allow fundamental discoveries in plant biology by elucidating transcriptional regulation, visualizing gene loci, identifying epigenetic modification and mechanisms regulating promoter activity et*C.* Multiplex genome editing, will make it possible for quick stacking of multiple traits and thus will improve breeding efficiency. The low cost, high precision and rapidity make genome editing a preferred plant breeding method for the future and more and more plants bred with CRISPR technology will be ready for marketing in the near future. Genome editing of crops is still marred in challenges in terms of off-targets. These can be circumvented by doing whole genome sequencing.

Whole genome sequencing (Islam et al. 2017; Sarkar et al. 2017) and moreover reference genome (Zhang et al. 2021a, b, c) of both the cultivated species would facilitate in applying site- directed genome editing which has great potential in mitigating abiotic stress (Biswas et al. 2021) as reported to be effective for drought in Arabidopsis (Paix˜ao et al. 2019) and maize (Shi et al. 2017) for salt in rice (Alfatih et al. 2020) and herbicide resistance in maize (Svitashev et al. 2015). ICAR-CRIJAF has initiated to develop an efficient method to introduce multiple site-directed point mutation in jute *EPSPS* and *ALS* gene using CRISPR/Cas9 to make jute tolerant to glyphosate and imazethapyr (Datta 2018).

6.5.3 RNA-Interference (RNAi) Technology

Inhibition of specific gene expression through RNAi (Fire et al. 1998) is also being used in many crop plants for boosting defense against various biotic/abiotic stresses in many plants (Saurabh et al. 2014). The micro RNAs having regulatory roles in stress response in plants by negatively affecting post-transcriptional gene expression have been identified like drought responsive miRNA in legume model plant, *Medicago truncatula* (Wang et al. 2011), one miRNA—miR393 up-regulated by high salinity, dehydration, cold in *Arabidopsis* (Sunkar and Zhu 2004) and two miRNAs—miR393 and miR169g up-regulated by dehydration in rice (Zhao et al. 2007).

Opportunity for application of RNAi in jute has been opened up with recent identification of 17 potential novel miRNA candidate in jute for the first time and their target genes with biochemical pathways analyzed through gene ontology and KEGG pathway analyses (Islam et al. 2015) which would help in further study on understanding the mechanisms of regulation of jute miRNA and its utilization in abiotic stress management. Furthermore, Hauqe et al. (2016) found that up-regulation of *miR319* with gene ATP-binding cassette (ABC) transport played a fundamental role as a metal transporter and increased As ($NaAsO_2$) stress tolerance in jute (*C. olitorius* cv. O 9897). In addition, the down-regulation of two miRNAs-*miR159* and *miR167*, and corresponding genes *ABC* and auxin responsive factor 8 (*ARF8*) in response to Mn ($KMnO_4$) and Cr ($K_2Cr_2O_7$), improved the remedial capacity of *C. olitorius* against Mn and Cr. Recently, in silico identification of conserved miRNA in *capsularis* jute genome through expressed sequence tags (EST) based homology search resulted in prediction of five potential miRNA belonging to five different mRNA families (miRNA1536, miRNA9567, miRNA 11,300, miRNA 8689). Functional annotation has identified 1052 gene targets—mostly being involved in developmental process including environmental response (Ahmed et al. 2021).

6.6 Novel Innovative Approach

6.6.1 Bio-designing Crassulacean Acid Metabolism for Improving Water Use Efficiency in Jute

Unique adaptation of crassulacean acid metabolism (CAM) plants is mediated via stomatal opening and CO_2-uptake at night when evapotranspiration rates are reduced, without any concomitant changes in leaf anatomy as in C_4 plants. Thus, CAM-mediated engineering of water use efficiency (WUE) in jute offers an exciting scope for sustaining agricultural production in semi-arid areas, avoiding competition of industrial crops like jute with food crops. Studying CAM plant and C_3 jute under water-deficit would allow the identification of the target genes in CAM pathways mediating C_3-to-CAM switching. Significant research efforts are now underway to harness the WUE of CAM by engineering this pathway into different crops. Recently RNAi has successfully been used to generate knock-out mutant lines for some CAM-related genes (Boxall et al. 2020). Even CRISPR/Cas9 mediated genome-editing has been used to generate mutation in CAM-related genes (Liu et al. 2019). CAM bio-designing of *A. thaliana* plant by overexpressing 13 enzymes and regulatory proteins of the core C4-metabolism cycle of CAM has also been reported (Lim et al. 2019). Genetic engineering with CAM abiotic stress-responsive TFs would be a promising strategy towards WUE and tolerance to abiotic stress in crop plants (Amin et al. 2019). Deshmukh and Murumkar (2013) studied the relation between nocturnal CO_2 uptake and WUE in a CAM weed *Commelina nudiflora*. The photosynthetic performance and CAM pathway in six *Coleus* spp. demonstrated the role of NADP-ME enzyme activity in photosynthetic efficiency (Ramanna and Chautanya 2015). The critical step for the understanding the mechanisms of bast fiber development and improving WUE is the sequencing of jute genome (Sarkar et al. 2017; Islam et al. 2017). Whole genome sequence of leading Indian *C. olitorius* variety, JRO 524 has been decoded with a total of 47,434 protein-coding genes and a total length of 209.4 Mb. The de novo hypocotyl transcriptomes of a white jute mutant of reduced lignin with higher cellulose has identified isoforms of several genes in the shikimate-aromatic amino acid and monolignol pathways and a cellulose synthase gene (Chakraborty et al. 2015). Nearly 15,000 genic SSR markers from bast transcriptome of *C. capsularis* cv. JRC 212 have also been developed (Saha et al. 2017; Satya et al. 2017). Genome sequencing coupled with hypocotyl transcriptome analyses has not only improved understanding of the molecular basis of major biosynthetic and signal transduction pathways in jute stress responses and bast fiber biogenesis, but also provides the platform for the application of genomic technologies for engineering the photosynthetic apparatus.

6.6.2 *Virus-Induced Gene Silencing (VIGS)*

Virus-induced gene silencing (VIGS) as both forward and reverse genetics technique is an effective tool for gene function analysis in various model plants as well as crop plants. With advancement in high-throughput transcript profiling to identify stress-responsive DEGs under various abiotic stresses, the application of VIGS is expected to be important for gene function analysis related to abiotic stresses (Ramegowda et al. 2014). In the recent past, functional characterization of genes associated with abiotic like drought in wheat (Manmathan et al. 2013; Kang et al. 2013), pea (Senthil-Kumar and Udayakumar 2006), barley (Liang et al. 2012), salt stress in chilli (Lee et al. 2010) through VIGS tool has successfully been accomplished.

In case of jute, plant induced with rice necrosis mosaic virus through sap inoculation showed higher biomass, faster growth, luxurious vigor, enhanced juvenility in place of any disease symptom (Ghosh 1982, 1995). Furthermore, the property of such virus-induced growth promotion in jute was also found to be epigenetic in nature (Ghosh 2002). Over-expression of genes under different functional categories like photosynthesis, plant growth and development, and membrane transport explained the virus-induced growth promotion phenomenon as well as the temporary passage of this property through seeds of inoculated plants of pigeon pea, rice bean, cotton and tomato (Ghosh et al. 2012). This indicates possibility of use of VIGS tools in abiotic stress resistance in jute.

6.7 Conclusion

Abiotic stress due to natural as well as anthropogenic activities alters the soil–plant-atmosphere continuum and adversely affects the crop growth and productivity. In combination these stresses make crop loss in jute up to 50% depending on degree of severity. Environmental friendly and resource sustainable technological intervention with multidimensional holistic approach is required to overcome all these stresses which would bring stability in fiber production. On the other hand, salinity tolerant jute varieties would expand the area under jute cultivation in large coastal area of both India and Bangladesh.

Advanced progress in the emerging field of omics including genomics, transcriptomics, proteomics, metabolomics along with recent reference genomes of both cultivated species of jute has opened up wide avenues towards challenge of addressing complex physiological, biochemical and genetic basis of plant response to various abiotic stresses as well mechanism of resistance. Integration of phenomics as reliable, automatic, sensor- and imaging-technology-based robotic, multifunctional, and high-throughput phenotyping platform with environmental monitoring for assaying multi-domain structural and functional dimension of a set of morphological, anatomical phenotypes under different abiotic stresses individually or in combination would accelerate the genomics approach particularly in increasing genetic gain and

improving selection efficiency in genomic selection where large genetic markers across the whole genome are used for selection through establishing the association of different stress induced phonotypic variation with genome-wide molecular marker taking the importance of genotype X environment interaction for genetic improvement for stress resistance.

Nanotechnology can be applied in mitigating the abiotic stress as a supplement to genomics approach as different nanoparticles have been proved to be effective in increasing root hydraulic conductance with efficient water uptake and enhancing ROS scavenging potential and anti-oxidant enzymatic activity in different crop plants like wheat (Jaberzadeh et al. 2013), flax (Aghdam et al. 2016), sorghum (Ahmed et al. 2011).

Genomics strategies for germplasm characterization particularly wild species and wild relatives would be more appropriate as genome variation in wild crop relatives would have direct relevance to breeding for abiotic stress tolerance. Comparative transcriptomics and metabolomics of populations of wild relatives growing in contrasting environments of stress and subsequent comparison with reference genome of cultivated species of jute will reveal the structural and functional dimension of genes conferring adaptation to different stresses. This strategy would be facilitated by recent advances in sequencing technologies and moreover, high quality reference genome sequence in jute as available. Large scale sequencing of germplasm including potential wild species will provide a platform to enable these approaches.

Application of insertional mutagenesis through transfer DNA (T-DNA), retroposons or transposable elements (TE) to dissect the functions of abiotic stress-related genes would be effective as jute genome is characterized by much higher proportion of retrotransposons (45.7%) with 5.5% DNA transposons (Sarkar et al. 2017) and TE has an important role in adaptation and TE insertion can affect host gene function and provides a mechanism for plant response to environmental stress.

However, instead of reductionism way of explaining individual factors responsible for a system, holism way of understanding a system in totality through integrating all individual factors is need of the hour to decipher the complexity of the abiotic stress—its effect on crop plant as well as plant responses to it. In this regard, plant systems biology approach integrating application of all omics—phenomics, genomics, transcriptomics, proteomics, metabolomics and epigenomics on a wide array of plant genetic resources (wild relatives, cultivar, landraces, obsolete varieties, germplasm accession, mutant resulted from physical and chemical mutagen, T-DNA and TE insertion) with available genome sequencing information would be very appropriate to discover gene network at both structural and functional level of plant responses and adaptive mechanisms to different abiotic stresses through both forward and reverse genetics technique. This would ultimately transform the concept and potential into reality of genomic improvement in jute for resistance to abiotic stresses.

References

Abdallah SB, Aung B, Amyot L, Lalin I, Lachâal M, Karray-Bouraoui N, Hannoufa A (2016) Salt stress (NaCl) affects plant growth and branch pathways of carotenoid and flavonoid biosyntheses in *Solanum nigrum*. Acta Physiol Plant 38:72. https://doi.org/10.1007/s11738-016-2096-8

Abubakari M, Moomin A, Nyarko G, Dawuda M (2017) Heavy metals concentrations and risk assessment of roselle and jute mallow cultivated with three compost types. Ann Agric Sci 62:145–150

Aghdam MTB, Mohammadi H, Ghorbanpour M (2016) Effects of nanoparticulate anatase titanium dioxide on physiological and biochemical performance of *Linum usitatissimum* (Linaceae) under well-watered and drought stress conditions. Braz J Bot 39:139–146

Ahmed DA, Slima DF (2018) Heavy metal accumulation by *Corchorus olitorius* L. irrigated with wastewater. Environ Sci Pollut Res 25:14996–15005

Ahmed G, Hossain ABMM, Islam MS (1989) Regeneration of multiple shoots in jute *Corchorus olitorius* (var. O-4) from cotyledon and hypocotyl explants from germinating seeds. Indian J Exp Biol 27:334–337

Ahmed M, Ahmed F, Ahmed J, Akhand MRN, Azim KF, Imran MAS, Hoque SF, Hasan M (2021) In silico identification of conserved miRNA in the genome of fibre biogenesis crop *Corchorus capsularis*. Heliyon 7:e06705. https://doi.org/10.1016/j.heliyon.2021.e06705

Ahmed M, Hassen F, Qadeer U, Aslam MA (2011) Silicon application and drought tolerance mechanism of sorghum. Afr J Agric Res 6:594–607

Ahmed R, Kabir SMT, Hossain MS, Ahmed B (2020) Whole transcriptome sequencing and analysis of jute (*Corchorus olitorius*) fiber cell. Biosci Agri Res 26:2204–2210

Akter J, Islam MS, Sajib AA, Ashraf N, Haque S, Khan H (2008) Microsatellite markers for determining genetic identities and genetic diversity among jute cultivars. Aust J Crop Sci 1:97–107

Alam MM, Sharmin S, Nabi Z, Mondal SI, Islam MS, Bin Nayeem S, Shoyaib M, Khan H (2010) A putative leucine-rich repeat receptor-like kinase of jute involved in stress response. Plant Mol Biol Rep 28:394–402

Alfatih A, Wu J, Jan SU, Zhang Z, Xia J, Xiang C (2020) Loss of rice *PARAQUAT TOLERANCE 3* confers enhanced resistance to abiotic stresses and increases grain yield in field. Plant Cell Environ 43:2743–2754

Aluka (2006–2021) African Plants. http://www.aluka.org. Accessed 31 March 2021

Amin AB, Rathnayake KN, Yim WC, Garcia TM, Wone B, Cushman JC, Wone BWM (2019) Crassulacean acid metabolism abiotic stress-responsive transcription factors: a potential genetic engineering approach for improving crop tolerance to abiotic stress. Front Plant Sci 10:129. https://doi.org/10.3389/fpls.2019.00129

Amin MN, Khatun A, Bhuiyan MSR, Sayed MA, Khandker SR (2012) Genetic transformation in white jute through *Agrobacterium* and salinity screening of transgenic plant. Bang J Agril Res 37:97–107

Arangzeb S (1988) Genetic resources of wild and weedy *Corchorus* and *Hibiscus* species and their value in breeding research. In: Denton IR (ed) Proceedings of the IJO/BJRI training course on collection, conversation and characterisation of jute and kenaf germplasm. International Jute Organization, Dhaka, Bangladesh

Arangzeb S, Khatun A (1980) A short note on interspecific hybridization between *Corchorus trilocularis* and *C. capsularis*. Bang J Jute Fibre Res 5:85–89

Armstrong W, Justin SHFW, Beckett PM, Lythe S (1991) Root adaptation to soil waterlogging. Aquati Bot 39:57–73

Banerjee SK, Mathew MD (1985) Carbonisation of jute stick, an agricultural waste. Agri Wastes 13:217–227

Basak M (1951) Behaviour of jute seeds towards saline solution during the process of germination. Sci Cult 17:38–40

Basu A, Ghosh M, Mayer R, Powell W, Basak SL, Sen SK (2004) Analysis of genetic diversity in cultivated jute determined by means of SSR markers and AFLP profiling. Crop Sci 44:678–685

Ben Yakoub AR, Tlahig S, Ferchichi A (2019) Germination, growth, photosynthesis and osmotic adjustment of tossa jute (*Corchorus olitrius* L.) seeds under saline irrigation. Polish J Environ Studies 28:935–942

Bhaduri SK, Haribabu M, Sen SK, Dasgupta PC, Mazumder P (1985) Constituents of jute seed. Indian J Agri Sci 55:750–753

Bharadwaj P, Beena MR, Sinha MK, Kirti PB (2011) In vitro regeneration and optimization of conditions for *Agrobacterium* mediated transformation in jute, *Corchorus capsularis*. J Plant Biochem Biotechnol 20:39–46

Bhattacharyya J, Chakraborty A, Roy S, Pradhan S, Mitra J, Chakraborty M, Manna A, Sikdar N, Chakraborty SSK (2015) Genetic transformation of cultivated jute (*Corchorus capsularis* L.) by particle bombardment using apical meristem tissue and development of stable transgenic plant. Plant Cell Tiss Org Cult 121:311–324

Biswas C, Dey P, Karmakar PG, Satpathy S (2015) Discovery of large-scale SNP markers and construction of linkage map in a RIL population of jute (*Corchorus capsularis*). Mol Breed 35:119

Biswas D, Chatterjee Saha S, Dey A (2021) CRISPR-Cas genome-editing tool in plant abiotic stress-tolerance. Plant Gene 26:100286

Bose TK (1976) Influence of temperature and photoperiod on growth, flowering and seed formation in jute. Indian J Agron 21:263–265

Boxall SF, Kadu N, Dever LV, Kneřová J, Waller JL, Gould PJD, Hartwell J (2020) *Kalanchoë* PPC1 is essential for crassulacean acid metabolism and the regulation of core circadian clock and guard cell signaling genes. Plant Cell 32:1136–1160

Brands SJ (ed) (1989–2021) Systema naturae 2000. In: The taxonomicon. Universal Taxonomic Services, Zwaag, The Netherlands. http://taxonomicon.taxonomy.nl/. Accessed 31 March 2021

Chakraborty A, Sarkar D, Satya P, Karmakar PG, Singh NK (2015) Pathways associated with lignin biosynthesis in lignomaniac jute fibres. Mol Genet Genom 290:1523–1542

Chakraborty K, Ghosh T (1969) Chaitali tossa—a new jute variety. Indian Farming 19:26–27

Changdee T, Morita S, Abe J, Ito K, Tajima R, Polthanee A (2008) Root anatomical responses to waterlogging at seedling stage of three cordage fiber crops. Plant Prod Sci 11:232–237

Changdee T, Polthanee A, Akkasaeng C, Morita S (2009) Effect of different waterlogging regimes on growth, some yield and roots development parameters in three fiber crops (*Hibiscus cannabinus* L., *Hibiscus sabdariffa* L. and *Corchorus olitorius* L.). Asian J Plant Sci 8:515–525

Chan-Navarrete R, Dolstra O, van Kaauwen M, van Bueren ETL, van der Linden CG (2016) Genetic map construction and QTL analysis of nitrogen use efficiency in spinach (*Spinacia oleracea* L.). Euphytica 208:621–636

Chaudhury BB, Basak SL (1969) Moisture stress and excess on cultivated jute. Indian J Agric Sci 39:666–671

Chen H, Chen M, Tao A, Zhang G, Xu J, Qi J, Fang P (2011) Construction of a molecular linkage map using SRAP and mapping of three qualitative traits in *Corchorus olitorius* L. Sci Agri Sin 44:2422–2430

Chen Q, Liu Z, Wang B, Wang X, Lai J, Tian F (2015) Transcriptome sequencing reveals the roles of transcription factors in modulating genotype by nitrogen interaction in maize. Plant Cell Rep 34:1761–1771

Chhapekar S, Raghavendrarao S, Pavan G, Ramakrishna C, Singh VK, Phanindra ML, Dhandapani G, Sreevathsa R, Ananda Kumar P (2015) Transgenic rice expressing a codon-modified synthetic CP4-EPSPS confers tolerance to broad-spectrum herbicide, glyphosate. Plant Cell Rep 34:721–731

Choudhary SB, Saha D, Sharma HK, Chowdhury I, Kumar A, Jambhulkar SK, Mitra J (2019) Transcriptional analysis of a delayed-flowering mutant under short-day conditions reveal genes related to photoperiodic response in tossa jute (*Corchorus olitorius* L.) Ind Crops Prod 132:476–486

Choudhary SB, Sharma HK, Karmakar PG, Saha AR, Hazra P, Mahapatra BS (2013) Nutritional profile of cultivated and wild jute (*Corchorus*) species. Aust J Crop Sci 7:1973–1982

Chowdhury SK, Das BB (2003) JRO 8432 (Shakti) tossa jute: a new high yielding variety for early sowing. Indian Farming 53:19–20
Curci PL, Cigliano RA, Zuluaga DL, Janni M, Sanseverino W, Sonnante G (2017) Transcriptomic response of durum wheat to nitrogen starvation. Sci Rep 7:1176. https://doi.org/10.1038/s41598-017-01377-0
Das M (1980) Utilization of jute stick in paper industry. Econ Pol Wkly 15:1679–1684
Das M, Banerjee S, Dhariwal R, Vyas S, Mir RR, Topdar N, Kundu A, Khurana JP, Tyagi AK, Sarkar D, Sinha MK, Balyan HS, Gupta PK (2012a) Development of SSR markers and construction of a linkage map in jute. J Genet 91:21–31
Das M, Banerjee S, Topdar N, Kundu A, Mir RR, Sarkar D, Sinha MK, Balyan HS, Gupta PK (2012b) QTL identification for molecular breeding of fibre yield and fibre quality traits in jute. Euphytica 187:175–189
Datta S (2016) Herbicide tolerance in jute. Annual report 2015–2016. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, pp 23–24
Datta S (2018) Herbicide tolerance in jute. Annual report 2016–2017. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, pp 14–15
Datta S, Mitra J, Saha D, Satya P, Anil Kumar A (2018) Genome-wide analysis of the aquaporin gene family in jute (*C. olitorius* and *C. capsularis).* Annual report 2017–2018. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India pp 13–14
Deshmukh RB, Murumkar CV (2013) Crassulacean acid metabolism (CAM) in leaves of a common weed *Commelina nudiflora* L. Intl J Plant Physiol Biochem 5:68–70
Dhar P, Ojha D, Kar CS, Mitra J (2018) Differential response of tossa jute (*Corchorus olitorius*) submitted to water deficit stress. Ind Crops Prod 112:141–150
Edmonds JM (1990) Herbarium survey of African *Corchorus* L. species. Systematic and ecogeographic studies on crop genepools no. 4. International Board for Plant Genetic Resources, Rome, p 284
Fageria NK, Baligar VC (2005) Enhancing nitrogen use efficiency in crop plants. Adv Agron 88:97–185
FAOSTAT (2021) Food and Agriculture Organization of the United Nations, Rome, Italy. www.fao.org/faostat/en/#home, Accessed 31 March 2021
Fartyal D, Agarwal A, James D, Borphukan B, Ram B, Sheri V, Agrawal PK, Achary VMM, Reddy MK (2018) Developing dual herbicide tolerant transgenic rice plants for sustainable weed management. Sci Rep 8:11598. https://doi.org/10.1038/s41598-018-29554-9
Fasinmirin JT, Olufayo AA (2009) Yield and water use efficiency of jute mallow *Corchorus olitorius* under varying soil water management strategies. J Med Plants Res 3:186–191
Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans.* Nature 391:806–811
Gelli M, Duo Y, Konda AR, Zhang C, Holding D, Dweikat I (2014) Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. BMC Genom 15:179. https://doi.org/10.1186/1471-2164-15-179
Ghorai AK, Bhattacharjee AK, Saha S, Rao PV, Bandopadhyay AK (2005) Impact of waterlogging on yield and quality of tossa jute (*Corchorus olitorius*). Indian J Agron 50:320–323
Ghorai AK, Bhattacharjee AK, Saha S, Rao PV (2003) Effect of waterlogging and drainage on yield and quality of jute (*Corchorus olitorius* and *C capsularis).* J Water Manage 11:68–73
Ghorai AK, Chakraborty AK, Pandit NC, Mandal RK (2006) Integrated weed management in jute (*Corchorus* spp. L.). Indian J Weed Sci 38:163–164
Ghosh RK, Phumichai T, Sreewongchai T, Nakasathien S, Phumichai C (2013) Evaluation of salt tolerance of jute (*Corchorus* spp.) genotypes in hydroponics using physiological parameters. Asian J Plant Sci 12:149
Ghosh SK (1982) Growth promotion in plant by rice necrosis mosaic virus. Planta 155:193–198
Ghosh SK (1995) Mechanism of rice necrosis mosaic virus-induced growth promotion and productivity in fibre crops: I. Physiological changes. Intl J Trop Plant Dis 13:221–236

Ghosh SK (2002) Rice necrosis mosaic virus technology—a new low cost production technology for jute fibre production. J Mycopathol Res 40:149–151

Ghosh SK, Acharyya S, Mir JI (2012) Passage of rice necrosis mosaic virus property induced growth promotion in some plants of commercial importance and its molecular evidence. Arch Phytopathol Plant Protect 45:2301–2323

Guttieri MJ, Frels K, Regassa T, Waters BM, Baenziger PS (2017) Variation for nitrogen use efficiency traits in current and historical great plains hard winter wheat. Euphytica 213:86–105

Han M, Wong J, Su T, Beatty PH, Good AG (2016) Identification of nitrogen use efficiency genes in barley: searching for QTLs controlling complex physiological traits. Front Plant Sci 7: 1587. https://doi.org/10.3389/fpls.2016.01587

Haque S, Ashraf N, Begum S, Sarkar RH, Khan H (2008) Construction of genetic map of Jute (*Corchorus olitorius* L.) based on RAPD markers. Plant Tiss Cult Biotechnol 18:165–172

Haque S, Begum S, Sarker RH, Khan H (2007) Determining genetic diversity of some jute varieties and accessions using RAPD markers. Plant Tiss Cult Biotechnol 17:183–191

Haque SA (2006) Salinity problems and crop production in coastal regions of Bangladesh. Pak J Bot 38:1359–1365

Hassan M, Dagari M, Babayo A (2016) Effect of citric acid on cadmium ion uptake and stress response of hydroponically grown jute mallow (*Corchorus olitorius*). J Environ Anal Toxicol 6:375

Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H, Ashikari M (2009) The ethylene response factors *SNORKEL*$_1$ and *SNORKEL*$_2$ allow rice to adapt to deep water. Nature 460:1026–1030

Hauqe S, Ferdous AS, Sarker SK, Islam MT, Hossain K, Khan H (2016) Identification and expression profiling of microRNAs and their corresponding targets related to phytoremediation of heavy metals in jute (*Corchorus olitorius* var. O-9897). Biores Comm 2:152–157

Heywood VH (1993) Flowering plants of the world. Oxford University Press, New York, p 335

Hossain ABMM, Ahmed G, Khan RI, Islam MS (1998) Transient GUS expression in jute (*Corchorus capsularis* var. D 154 explants after infection by *Agrobacterium tumefaciens*. Plant Tiss Cult 8:11–18

Hossain MB, Awal A, Rahman MA, Haque S, Khan H (2003) Distinction between cold sensitive and cold tolerant jute by DNA polymorphisms. J Biochem Mol Biol 36:427–432

Hossain MB, Haque S, Khan H (2002) DNA fingerprinting of jute germplasm by RAPD. J Biochem Mol Biol 35:414–419

Islam AS (1964) A rare hybrid combination through application of hormone and embryo culture. Nature 201:320

Islam AS, Haque M (1967) A natural *Corchorus* species hybrid not possible to produce artificially. Sind Univ Res J 2:191–198

Islam AS, Rashid A (1960) First successful hybrid between the two jute-yielding species, *Corchorus olitorius* L. (Tossa) x *C. capsularis* L. (White). Nature 185:258–259

Islam M, Begum M, Islam M (2011) Screening of jute mutants for salinity tolerance. Intl J Sustain Crop Prod 6:6–11

Islam MR, Khan MH, Zohra FT, Hossain MB, Seraj Z (1999) Stable transformation of jute (*Corchorus capsularis* L. var. CVL-1) calli and high efficiency marker gene insertion in explants. Plant Tiss Cult 9:35–43

Islam MS, Azam MS, Sharmin S, Sajib AA, Alam MM, Reza MS, Ahmed R, Khan H (2013) Improved salt tolerance of jute plants expressing the *katE* gene from *Escherichia coli*. Turk J Biol 37:206–211

Islam MS, Saito JA, Emdad EM, Ahmed B, Islam MM, Halim A, Hossen QMM, Hossain MZ, Ahmed R, Hossain MS, Kabir SMT, Khan MSA, Khan MM, Hasan R, Aktar N, Honi U, Islam R, Rashid MM, Wan X, Hou S, Haque T, Azam MS, Moosa MM, Elias SM, Hasan AMM, Mahmood N, Shafiuddin M, Shahid S, Shommu NS, Jahan S, Roy S, Chowdhury A, Akhand AI, Nisho GM, Uddin KS, Rabeya T, Hoque SME, Snigdha AR, Mortoza S, Matin SA, Islam MK, Lashkar MZH, Zaman M, Yuryev A, Uddin MK, Rahman MS, Haque MS, Alam MM, Khan H,

Alam M (2017) Comparative genomics of two jute species and insight into fibre biogenesis. Nat Plants 3:16223–16229
Islam MT, Ferdous AS, Najnin RA, Sarker SK, Khan H (2015) High-throughput sequencing reveals diverse sets of conserved, nonconserved, and species-specific miRNAs in jute. Intl J Genom 2015:1–14
Jaberzadeh A, Moaveni P, Moghadam HRT, Zahedi H (2013) Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. Notulae Botanicae Horti Agrobotanici Cluj Napoca 41:201–207
Jackson MB, Armstrong W (1999) Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. Plant Biol 1:274–287
Jatothu JL, Kumar AA, Choudhary SB, Sharma HK, Maruthi RT, Kar CS, Mitra J (2018) Genetic diversity analysis in tossa Jute (*Corchorus olitorius* L.) germplasm lines. J Appl Nat Sci 10:1–3
Johansen C, Waseque M, Begum S (1985) Effect and interaction of photoperiod, temperature, water stress and nitrogen on flowering and growth in jute. Field Crops Res 12:397–406
Joseph J, Saha A (1978) Photoperiod insensitivity in jute. Indian J Genet 38:313–317
Jui SA, Mukul MM, Rashid MHO, Nur IJ, Ghosh RK, Mostofa MG, Akter N, Sultan MT (2021) Responses and screening of white jute (*Corchorus capsularis* L.) genotypes against salinity stresses. Plant Sci Today 8:416–424
Kang G, Li G, Ma H, Wang C, Guo T (2013) Proteomic analysis on the leaves of *TaBTF3* gene virus-induced silenced wheat plants may reveal its regulatory mechanism. J Proteom 83:130–143
Kar BK, De Sarkar BK (1957) Investigations of salt tolerance in jute (*Corchorus*). In Proceedings of the 44th Indian Science Congress Association, 14–20 Jan, 1957, University of Calcutta, Calcutta, p 386
Kar BK, De Sarkar BK (1961) Investigations on the physiology of jute V. Growth and development of root system in *Corchorus capsularis* and *C. olitorius*. Indian J Plant Physiol 4:11–20
Kar CS, Satya P, Mitra J, Sarkar D, Sinha MK, Kundu A, Mahapatra BS (2010) Varietal development of jute and allied fibres in India. Indian Farming 60:5–9
Karim M, Rahman SM, Ahmed F, Patwary SU (1972) Studies on the efficiency of nitrogen and phosphorus fertilizer utilization by jute under various methods and times of application using isotopes. Geoderma 7:121–131
Karmakar PG, Mahapatra, AK, Saha A, Gupta D, Choudhary SB, Sharma HK (2014) Characterization of tossa jute (*Corchorus olitorius* L.) germplasm. ICAR-Central Research Institute for jute and Allied Fibres, Barrackpore, India, p 66
Khan MSY, Bano S, Javed K, Mueed MA (2006) A comprehensive review on the chemistry and pharmacology of *Corchorus* species—a source of cardiac glycosides, triterpenoids, ionones, flavonoids, coumarins, steroids and some other compounds. J Sci Ind Res 65:283–298
Khandker S, Begum H, Alam AKMM, Alam MM, Ahmad SA, Iqbal MS, Islam MM (1992) Salinity effect on germination and seedling growth of seeds of different species of jute (*Corchorus* spp.) and allied fibre (*Hibiscus* spp.) in Bangladesh. Bang J Jute Fiber Res 17:85–91
Khanom A, Chowdhury AK, Mehedi MNH, Mohosina F, Akhter M (2018) Growth and yield performances of salt tolerant jute lines (*Corchorus capsularis* L.) in coastal area of Bangladesh. Bang J Environ Sci 35:51–56
Khatun A, Saha CK, Naher Z, Mahbub S, Siddique AB, Bilkis S (2003) Plant Regeneration from the cotyledons of tossa jute (*Corchorus olitorius* L.). Biotechnol 2:206–213
Kumar AA, Maruthi RT, Mondal K, Gotyal BS (2019) Wide hybridization in jute. Annual report 2019. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, p 10
Kumar D, Begum T, Kotal B, Shil S (2008) Descriptors of Jute Varieties. ICAR-Central Rersearch Institute for Jute and Allied Fibres, Barrackpore, India
Kumar M, Ghorai AK, Mitra S, Kundu DK (2013) Major weed flora in jute and their managemnent. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India
Kumar S, Shamna A, Roy ML, Jha SK (2017) Impact of herbicide application on fibre yield of jute (*Corchorus* spp.) in West Bengal. Intl J Sci Environ Technol 6:1360–1366

Kumari N, Choudhary SB, Sharma HK, Singh BK, Kumar AA (2018) Health-promoting properties of *Corchorus* leaves: a review. J Herbal Med. https://doi.org/10.1016/j.hermed.2018.10.005

Kundu A, Chakraborty A, Mandal NA, Das D, Karmakar PG, Singh NK, Sarkar D (2015) A restriction-site-associated DNA (RAD) linkage map, comparative genomics and identification of QTL for histological fibre content coincident with those for retted bast fibre yield and its major components in jute (*Corchorus olitorius* L., Malvaceae s. l.). Mol Breed 35:19

Kundu A, Topdar N, Sarkar D, Sinha MK, Ghosh A, Banerjee S, Das M, Balyan HS, Mahapatra BS, Gupta PK (2013) Origins of white (*Corchorus capsularis* L.) and dark (*C. olitoriusis* L.) jute: a reevaluation based on nuclear and chloroplast microsatellites. J Plant Biochem Biotechnol 22:372–381

Kundu BC (1944) Anatomy of jute stem with special reference to cambial activity and distribution of fibres in relation to leaf trace system. J Asiatic Soc Bengal (Sci) 10:27–52

Kundu BC (1951) Origin of jute. Indian J Genet 2:95–99

Kundu BC (1956) Jute—world's foremost fibre I. Botany, agronomy, diseases and pests. Econ Bot 10:103–133

Kundu BC, Basak KC, Sarkar PB (1959) Jute in India. The Indian Central Jute Committee, Calcutta, India

Kuroha T, Nagai K, Gamuyao R, Wang DR, Furuta T, Nakamori M, Kitaoka T, Adachi K, Minami A, Mori Y, Mashiguchi K, Seto Y, Yamaguchi S, Kojima M, Sakakibara H, Wu J, Ebana K, Mitsuda N, Ohme-Takagi M, Yanagisawa S, Yamasaki M, Yokoyama R, Nishitani K, Mochizuki T, Tamiya G, McCouch SR, Ashikari M (2018) Ethylene-gibberellin signaling underlies adaptation of rice to periodic flooding. Science 361:181–186

Lee SC, Choi DS, Hwang IS, Hwang BK (2010) The pepper oxidoreductase *CaOXR1* interacts with the transcription factor *CaRAV1* and is required for salt and osmotic stress tolerance. Plant Mol Biol 73:409–424

Liang J, Deng G, Long H, Pan Z, Wang C, Cai P, Xu D, Nima ZX, Yu M (2012) Virus-induced silencing of genes encoding LEA protein in Tibetan hulless barley (*Hordeum vulgare* ssp. *vulgare*) and their relationship to drought tolerance. Mol Breed 30:441–451

Lim SD, Lee S, Choi WG, Yim WC, Cushman JC (2019) Laying the foundation for crassulacean acid metabolism (CAM) biodesign: expression of the C_4 metabolism cycle genes of CAM in *Arabidopsis*. Front Plant Sci 10:101. https://doi.org/10.3389/fpls.2019.00101

Liu D, Chen M, Mendoza B, Cheng H, Hu R, Li L, Trinh CT, Tuskan GA, Yang X (2019) CRISPR/Cas9-mediated targeted mutagenesis for functional genomics research of crassulacean acid metabolism plants. J Exp Bot 70:6621–6629

Liu Z, Zhu C, Jiang Y, Tian Y, Yu J, An H, Tang W, Sun J, Tang J, Chen G, Zhai H, Wang C, Wan J (2016) Association mapping and genetic dissection of nitrogen use efficiency-related traits in rice (*Oryza sativa* L.). Funct Integr Genom 16:323–333. https://doi.org/10.1007/s10142-016-0486-z

Ma H, Wang R, Wang X, Ma H (2009) Identification and evaluation of salt tolerance of jute germplasm during germination and seedling periods. J Plant Genet Resour 10:236–243

Ma H, Yang R, Wang Z, Yu T, Jia Y, Gu H, Wang X, Ma H (2011) Screening of salinity tolerant jute (*Corchorus capsularis* and *C. olitorius*) genotypes via phenotypic and phsiology-assisted procedures. Pak J Bot 43:2655–2660

Mahapatra AK, Saha A (2008) Genetics resources of jute and allied fibre crops. In: Karmakar PG, Hazra SK, Ramasubramanian T, Mandal RK, Sinha MK, Sen HS (eds) Jute and allied fibre updates: production and technology. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, pp 18–37

Mahapatra AK, Saha A, Basak SL (1998) Origin, taxonomy and distribution of *Corchorus* species in India. Green J 1:64–82

Mahapatra AK, Saha A, Gupta D (2006) Catalogue on evaluation of white jute germplasm (*Corchorus capsularis* L). ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, p 45

Maity S, Datta AK (2008) Cytomorphological studies in F_1 hybrids (*Corchorus capsularis* L. x *Corchorus trilocularis* L.) of jute (Tiliaceae). Compar Cytogenet 2:143–149

Majumder S, Datta K, Sarkar C, Saha SC, Datta SK (2018) The development of *Macrophomina phaseolina* (Fungus) resistant and glufosinate (herbicide) tolerant transgenic jute. Front Plant Sci 9:920. https://doi.org/10.3389/fpls.2018.00920

Manmathan H, Shaner D, Snelling J, Tisserat N, Lapitan N (2013) Virus-induced gene silencing of *Arabidopsis thaliana* gene homologues in wheat identifies genes conferring improved drought tolerance. J Exp Bot 64:1381–1392

Mathur M, Sundaramoorthy S (2008) Distribution pattern and growth assessment of *Corchorus depressus* in semi-arid Indian desert. Trop Ecol 49:69–71

Mazen A (2004) Accumulation of four metals in tissues of *Corchorus olitorius* and possible mechanisms of their tolerance. Biol Plant 48:267–272

Mia MM, Shaikh AQ (1967) Gamma radiation and interspecific hybridization in jute (*Corchorus capsularis* L. and *C. olitorius* L.). Euphytica 16:16–68

Mir JI, Karmakar PG, Chattopadhyay S, Chaudhury SK, Ghosh SK, Roy A (2008a) SSR and RAPD profile based grouping of selected jute germplasm with respect to fibre fineness trait. J Plant Biochem Biotechnol 17:29–35

Mir RR, Rustgi S, Sharma S, Singh R, Goyal A, Kumar I, Gaur A, Tyagi AK, Khan H, Sinha MK, Balyan HS, Gupta PK (2008b) A preliminary genetic analysis of fibre traits and the use of new genomic SSRs for genetic diversity in jute. Euphytica 161:413–427

Mir RR, Banerji S, Das M, Gupta V, Tyagi AK, Sinha MK, Balyan HS, Gupta PK (2009) Development and characterization of large-scale simple sequence repeats in jute. Crop Sci 49:1687–1694

Mitra J (2001) Genetics and genetic improvement of drought tolerance in crop plants. Curr Sci 80:758–762

Mitra S, Mitra J, Satya P, Saha D, Kumar M (2016) Enhancement of nitrogen use efficiency in jute. Annual report 2015–2016. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, p 29

Mukul MM, Ahmed SSU, Akter N, Mostofa MG, Rahman MS, Talukder FU (2021) Responses of seed germination, seedling growth under salinity stresses and variability for phenotypic traits in tossa jute (*Corchorus olitorius* L.). Plant Sci Today 8:166–180

Mukul MM, Akter N, Mostofa MG, Ahmed SSU, Nur IJ, Al-Mamun M, Rashid MH (2020a) Analyses of genetic variability, character association, heritability and genetic advance of tossa jute (*Corchorus olitorius*) genotypes for morphology and stem anatomy. Am J BioSci 8:99–112

Mukul MM, Akter N, Mostofa MG, Rahman MS, Hossain MA, Roy DC, Jui SA, Karim MM, Ferdush J, Hoque MM, Mollah MAF (2020b) Analyses of variability, euclidean clustering and principal components for genetic diversity of eight tossa jute (*Corchorus olitorius* L.) genotypes. Plant Sci Today 7:564–576

Naher Z, Khatun A, Mahbub S, Alim MA, Siddique AB (2003) Influence of genotypes on plant regeneration from cotyledons of *C. capsularis* L. Biotechnology 2:44–51

Naik MR, Barman D, Maruthi RT, Mandal UK, Kundu DK (2015) Screening of tossa jute (*Corchorus olitrius* L.) varieties against salt stress. Ecoscan 9:815–819

Naik MR, Barman D, Maruthi RT, Babu VR, Mandal UK, Kundu DK (2019) Assessment of salinity tolerance based upon morpho-physiological attributes in white jute (*Corchorus capsularis* L.). J Environ Biol 40:377–383

Negm S, EI-Shabrawy O, Arbid M, Radwan AS (1980). Toxicological study of the different organs of *Corchorus olitorius* L. plant with special reference to their cardiac glycosides content. Z Ernaehrungsw 19:28–32

Ngomuo M, Stoilova T, Feyissa T, Kassim N, Ndakidemi PA (2017) The genetic diversity of leaf vegetable jute mallow (*Corchorus* spp.): a review. Indian J Agri Res 51:405–412

Niyitanga S, Yao J, Ibrahim AK, Afzal MZ, Chen S, Zhang L (2021) Development of Genome-wide SSR markers for physical map construction with PCR-based polymorphic SSRs in jute (*Corchorus* spp.). Tropical Plant Biol. https://doi.org/10.1007/s12042-021-09301-7

Nyadanu D, Amoah RA, Kwarteng AO, Akromah R, Aboagye LM, Adu-Dapaah H, Dansi A, Lotsu F, Tsama A (2016) Domestication of jute mallow (*Corchorus olitorius* L.): ethnobotany,

production constraints and phenomics of local cultivars in Ghana. Genet Resour Crop Evol 63:1–17
Odunnaike JO, Ajiboye AA, Agboola DA (2013) Investigations on the effect of salinity stress on the seedling growth of *Corchorus olitorius* (L.). Sci Focus 18:21
Ogunkunle CO, Ziyath AM, Adewumi FE, Fatoba PO (2015) Bioaccumulation and associated dietary risks of Pb, Cd, and Zn in amaranth (*Amaranthus cruentus*) and jute mallow (*Corchorus olitorius*) grown on soil irrigated using polluted water from Asa River Nigeria. Environ Monit Assess 187:281
Okoegwale EE, Olumese GO (2001) Folk medicine practices in Nigeria: some medicinal plants of Esan people in Edo State Nigeria. Niger J Appl Sci 4:2350–2358
Paixão JFR, Gillet FX, Ribeiro TP, Bournaud C, Lourenco-Tessutti IT, Noriega DD, de Melo BP, de Almeida-Engler J, Grossi-de-Sa MF (2019) Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a histone AcetylTransferase. Sci Rep 9:8080. https://doi.org/10.1038/s41598-019-44571-y
Palit P, Bhattacharyya AC (1981) Germination and water uptake of jute seeds under water stress. Indian J Exp Biol 19:848–852
Palit P, Bhattacharyya AC (1984) Characterization of the type of photosynthetic carbon dioxide fixation in jute (*Corchorus olitorius* L.). J Exp Bot 35:169–173
Palit P, Sasmal BC, Bhattacharyya AC (1996) Germplasm diversity and estimate of genetic advance of four morphophysiological traits in a world collection of jute. Euphytica 90:49–58
Palit P, Singh VK (1991) External water potential as the effector for proline accumulation in imbibing jute (*Corchorus capsularis* L.) seeds. Indian J Exp Biol 29:793–794
Palve SM, Sinha MK (2005) Genetic variation and interrelationships among fibre yield attributes in secondary gene pool of *Corchorus* spp. SABRAO J Breed Genet 37:55–64
Palve SM, Sinha MK, Chattopahdyay S (2004) Genetic variability for fibre strength and fineness in wild relatives of genus *Corchorus*. In: Proceedings of national seminar on diversified uses of Jute and allied fibre crops, Barrackpore, Kolkata, India, pp 99–103
Pan J, Sharif R, Xu X, Chen X (2021) Mechanisms of waterlogging tolerance in plants: research progress and prospects. Front Plant Sci. https://doi.org/10.3389/fpls.2020.627331
Pandey SK, Maruthi RT, Alam NM, Mitra S, Satya P, Kar CS, Mitra J (2020) Jute varieties in India—an overview. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, p 60
Parvin D, Rashid P, Karmoker J (2018) Anatomical responses of jute (*Corchorus capsularis* L. cv. D-154) to waterlogging. Dhaka Univ J Biol Sci 27:213–219
Patel CS, Mandal AK (1983) Effect of moisture regimes and level of fertilizer application on yield and water requirement of jute (*Corchorus olitorius* L. and *C. capsularis* L). J Agric Sci 101:311–316
Prodhan AKMA, Rahman ML, Haque MA (2001a) Effects of water stresses on growth attributes in jute I. Plant height. Pak J Biol Sci 4:128–135
Prodhan AKMA, Rahman ML, Haque MA (2001b) Effects of water stresses on growth attributes in jute II. Plant base diameter. Plant height. Pak J Biol Sci 4:660–664
Qi JM, Zhou DX, Wu WR, Lin LH, Fang PP, Wu JM (2003a) The application of RAPD technology in genetic diversity detection of Jute. Yi Chuan Tai Xue Bao 30(10):926–932
Qi JM, Zhou DX, Wu WR, Lin LH, Wu JM, Fang PP (2003b) Application of ISSR technology in genetic diversity detection of jute. Ying Yong Sheng Tai Xue Bao 14(9):1473–1477
Qi JM, Zhou DX, Wu WR, Lin LH, Fang PP, Wu JM (2004) A comparison between RAPD and ISSR technology in detection of genetic diversity of jute. Sci Agri Sin 37:2006–2011
Ramanna GV, Chautanya KV (2015) Variations in δ 13C rates and crassulacean acid metabolism of six *Coleus* species. Brit J Appl Sci Technol 6:295–303
Ramegowda V, Mysore KS, Senthil-Kumar M (2014) Virus-induced gene silencing is a versatile tool for unraveling the functional relevance of multiple abiotic-stress-responsive genes in crop plants. Front Plant Sci 5:62–74
Rao PV (1979) Analysis of growth-environment relationship in jute. Bound-Layer Meteorol 41:1–8

Roy A, Bandyopadhyay A, Mahapatra AK, Ghosh SK, Singh NK, Bansal KE, Koundal KR, Mohapatra T (2006) Evaluation of genetic diversity in jute (*Corchorus* species) using STMS, ISSR and RAPD markers. Plant Breed 125:292–297

Roy Chowdhury S, Choudhuri MA (1985) Hydrogen peroxide metabolism as an index of water stress tolerance in jute. Physiol Planta 65:503–507

Roy Chowdhury S, Choudhyri MA (1986) Effects of calcium ions on responses of two jute species under water-deficit stress. Physiol Planta 68:86–92

Ryan GF (1970) Resistance of common groundsel to simazine and atrazine. Weed Sci 18:614–616

Saha CK, Sagorika S (2013) Carbon credit of jute and sustainable environment. Jute Matters 1:1–2

Saha D, Rana RS, Chakraborty S, Datta S, Kumar AA, Chakraborty AK, Karmakar PG (2017) Development of a set of SSR markers for genetic polymorphism detection and interspecific hybrid jute breeding. Crop J 5:416–429

Saha P, Datta K, Majumder S, Sarkar C, China SP, Sarkar SN, Sarkar D, Datta SK (2014) *Agrobacterium* mediated genetic transformation of commercial jute cultivar *Corchorus capsularis* cv. JRC 321 using shoot tip explants. Plant Cell Tiss Org Cult 118:313–326

Saleem MH, Ali S, Rehman M, Hasanuzzaman M, Rizwan M, Irshad S, Shafiq F, Iqbal M, Alharbi BM, Alnusaire TS, Qari SH (2020) Jute: a potential candidate for phytoremediation of metals—a review. Plants 9:258. https://doi.org/10.3390/plants9020258

Saleem MH, Fahad S, Khan SU, Ahmar S, Khan MHU, Rehman M, Maqbool Z, Liu L (2019) Morpho-physiological traits, gaseous exchange attributes, and phytoremediation potential of jute (*Corchorus capsularis* L.) grown in different concentrations of copper-contaminated soil. Ecotoxicol Environ Saf 189:109915

Samad MA, Kabir G, Islam AS (1992) Interphase nuclear structure and heterochromatin in two species of *Corchorus* and their F_1 hybrid. Cytologia 57:21–25

Saraswat VN (1999) Weed management in jute and jute based cropping system. In: Palit P, Pathak S, Singh DP (Ed) Jute and allied fibres: agriculture and processing. ICAR0-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, pp 193–200

Sarkar D (2019) Advanced breeding in jute: fertile sexual hybrid of the two cultivated jute species (*Corchorus capsularis* x *Corchorus olitorius*) annual report 2019. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, India, pp 1–2

Sarkar D, Kundu A, Saha A, Mondal NA, Sinha MK, Mahapatra BS (2011) First nuclear DNA amounts in diploid ($2n = 2x = 14$) *Corchorus* spp. by flow cytometry: genome sizes in the cultivated jute species (*C. capsularis* L. and *C. olitorius* L.) are ~300% smaller than the reported estimate of 1100–1350 Mb. Caryologia 64:147–153

Sarkar D, Mahato AK, Satya P, Kundu A, Singh S, Jasawal PK, Singh A, Bahadur K, Pattnaik S, Singh N, Chakraborty A, Mandal NA, Das D, Basu T, Sevanthi AS, Saha D, Datta S, Kar CS, Mitra J, Datta K, Karmakar PG, Sharma TR, Mohapatra T, Singh NK (2017) The draft genome of *Corchorus olitorius* cv. JRO 524 (Navin). Genomics Data 12:151–154

Sarker RH, Al-Amin GM, Hassan F, Hoque MI (2008) Agrobacterium-mediated genetic transformation of two varieties of jute (*Corchorus capsularis* L.). Plant Tiss Cult Biotechnol 18:7–16

Satya P, Chakraborty A, Jana S, Majumdar S, Karan M, Sarkar D, Datta S, Mitra J, Kar CS, Karmakar PG, Singh NK (2017) Identification of genic SSRs in jute (*Corchorus capsularis* L., Malvaceae) and development of markers for phenylpropanoid biosynthesis genes and regulatory genes. Plant Breed 136:784–797

Saurabh S, Vidyarthi AS, Prasad D (2014) RNA interference: concept to reality in crop improvement. Planta 239:543–564

Sawarkar A, Yumnam S, Mukherjee S, Sarkar KK (2016) Screening for moisture stress tolerant genotypes in *C. olitorius* on the basis of seedling characters. Indian J Plant Genet Resour 29:170–176

Sengupta JC, Sen NK (1946) Photoperiodism in jute. Nature 153:655–656

Sengupta JC (1947) Variation of osmotic value in jute plants. J Indian Bot Soc 26:157–175

Sengupta JC, Sen G (1952) Further investigations on the photoperiodic effect in jute. Indian J Agric Sci 22:1–32
Sengupta JC, Sen NK (1947) Investigation on the early flowering of jute (*Corchorus olitorius* L) sown in March. J Indian Bot Soc 26:41–49
Sengupta JC, Sen NK (1944) On photoperiodic effect on jute. Indian J Agric Sci 14:196–202
Senthil-Kumar M, Udayakumar M (2006) High-throughput virus-induced gene-silencing approach to assess the functional relevance of a moisture stress-induced cDNA homologous to lea4. J Exp Bot 57:2291–2302
Seraj Z, Sarker AB, Islam AS (1992) Plant regeneration in a jute species (*Corchorus capsularis*) and its possible relationship with glyoxalase-I. Plant Cell Rep 12:29–33
Shah SS, Shaikh MN, Khan MY, Alfasane MA, Rahman MM, Aziz MA (2021) Present status and future prospects of jute in nanotechnology: a review. Chem Record 21:1631–1665
Shanhua T, Benor S, Blattner FR, Demissew S, Karl H (2010) Collection and ethnobotanical investigation of *Corchorus* species in Ethiopia: potential leafy vegetables for dry regions. Genet Resour Crop Evol 57:293–306
Sharma HK, Satya P, Choudhary SB, Kar CS, Mitra J, Karmakar PG (2012) Determination of threshold and screening for salt tolerance in jute. Indian J Agri Res 46:324–330
Sharmin S, Moosa MM, Islam MS, Kabir I, Akter A, Khan NH (2011) Identification of a novel dehydration responsive transcript from tossa jute (*Corchorus olitorius*). J Cell Mol Biol 9:21–29
Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE (2017) ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207–216
Singh AK (2017) The potential of jute crop for mitigation of greenhouse gas emission in the changing climatic scenario. Intl J Agri Sci 13:419–423
Singh J, Sharma A, Sharma P, Singh S, Das D, Chawla G, Singha A, Nain L (2020) Valorization of jute (*Corchorus* sp.) biomass for bioethanol production. Biomass Conv Bioref. https://doi.org/10.1007/s13399-020-00937-1
Sinha MK, Mitra S, Nandy S, Sengupta D, Dutta P, Das F, Chakrabarty SC (2004) Interspecific hybrid between two jute (*Corchorus*) species for textile quality fibre. Indian J Genet 64:310–314
Sultana N, Khan H, Ashraf N, Sharkar MTK (2006) Construction of an intraspecific linkage map of jute. Asian J Plant Sci 5:758–762
Sun Y, Zhang X, Wu C, He Y, Ma Y, Hou H, Guo X, Du W, Zhao Y, Xia L (2016) Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate synthase. Mol Plant 9:628–631
Sunkar R, Zhu JK (2004) Novel and stress-regulated micro RNAs and other small RNAs from Arabidopsis. Plant Cell 16:2001–2019
Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM (2015) Targeted mutagenesis, precise gene editing and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiol 169:931–945
Swaminathan M, Iyer RD (1961) Skewed recombination in a rare interspecific jute hybrid. Nature 192:893–894
Taneenah A, Nulit R, Yusof UK, Janaydeh M (2015) Tolerance of Molokhia (*Corchorus olitorius* L.) seed with dead sea water, sea water, and NaCl: Germination and anatomical approach. Adv Environ Biol 9:106–116
Tao A, Huang L, Wu G, Afshar RK, Qi J, Xu J, Fang P, Lin L, Zhang L, Lin P (2017) High-density genetic map construction and QTLs identification for plant height in white jute (*Corchorus capsularis* L.) using specific locus amplified fragment (SLAF) sequencing. BMC Genomics 18:355
Topdar N, Kundu A, Sinha MK, Sarkar D, Das M, Banerjee S, Kar CS, Satya P, Balyan HS, Mahapatra BS, Gupta PK (2013) A complete genetic linkage map and QTL analyses for bast fibre quality traits, yield and yield components in jute (*Corchorus olitorius* L.). Cytol Genet 47:129–137

Uddin NM, Wahid-U-Zzaman M, Rahman MM, Kim JE (2016) Phytoremediation potential of kenaf (*Hibiscus cannabinus* L.), mesta (*Hibiscus sabdariffa* L.) and jute (*Corchorus capsularis* L.) in arsenic-contaminated Soil. Kor J Environ Agri 35:111–120

Wahab MA (1978) Influence of waterlogging on the yield of *capsularis* jute. In: Proceedings of the 3rd Bangladesh Science Conference, (BSC78), Bangladesh, pp 14–15

Wang T, Chen L, Zhao M, Tian Q, Zhang WH (2011) Identification of drought-responsive microRNAs in *Medicago truncatula* by genome-wide high-throughput sequencing. BMC Genomics 12:367

Wild H (1984) Tiliaceae. In: Leistner OA (ed) Flora of Southern Africa 21(1). Botanical Research Institute, Department of Agriculture, Pretoria, South Africa, pp 30–44

Xu X, Wang H, Qi X, Xu Q, Chen X (2014) Waterlogging-induced increase in fermentation and related gene expression in the root of cucumber (*Cucumis sativus* L.). Sci Hort 179:388–395. https://doi.org/10.1016/j.scienta.2014.10.001

Yang X, Nian J, Xie Q, Feng J, Zhang F, Jing H, Zhang J, Dong G, Liang Y, Peng J, Wang G, Qian Q, Zuo J (2016) Rice ferredoxin-dependent glutamate synthase regulates nitrogen-carbon metabolomes and is genetically differentiated between japonica and indica subspecies. Mol Plant 9:1520–1534

Yang Z, Dai Z, Chen X, Xie D, Tang Q, Cheng C, Xu Y, Deng C, Liu C, Chen J, Su J (2020a) Gene coexpression network analysis and tissue-specific profiling of gene expression in jute (*Corchorus capsularis* L.). BMC Genomics 21:406. https://doi.org/10.1186/s12864-020-06805-6

Yang Z, Dai Z, Lu R, Wu B, Tang Q, Xu Y, Cheng C, Su J (2017) Transcriptome analysis of two species of jute in response to polyethylene glycol (PEG)-induced drought stress. Sci Rep 7:16565

Yang Z, Dai Z, Xie D, Chen J, Tang Q, Cheng C, Xu Y, Wang T, Su J (2018) Development of an InDel polymorphism database for jute via comparative transcriptome analysis. Genome 61:323–327

Yang Z, Wu Y, Dai Z, Chen X, Wangb H, Yang S, Xie D, Tang Q, Cheng C, Xu Y, Deng C, Liu C, Chen J, Su J (2020b) Comprehensive transcriptome analysis and tissue-specific profiling of gene expression in jute (*Corchorus olitorius* L.). Ind Crops Prod 146:112101

Yang Z, Yang Y, Dai Z, Xie D, Tang Q, Cheng C, Xu Y, Liu C, Deng C, Chen J, Su J (2019) Construction of a high-resolution genetic map and identification of quantitative trait loci for salt tolerance in jute (*Corchous* spp.). BMC Plant Biol 19:391–400

Yumnam S, Sawarkar A, Mukherjee S, Sarkar K (2016) Assessment of genetic diversity in jute (*Corchorus olitorius* L.) under rainfed condition. Elec J Plant Breed 7:454–458

Zhang G, Huang S, Zhang C, Wu Y, Li D, Deng J, Shan S, Qi J (2021a) Overexpression of CcNAC1 gene promotes early flowering and enhances drought tolerance of jute (*Corchorus capsularis* L.). Protoplasma 258:337–345

Zhang G, Huang S, Zhang C, Wu Y, Li D, Deng J, Shan S, Qi J (2021b) Comparative transcriptome sequencing analysis and functional identification of a NAM-2-like gene in jute (*Corchorus capsularis* L.). Plant Physiol Biochem 161:25–35

Zhang L, Gao Z, Wan X, Xu Y, Zhang L, Tao A, Fang P, Qi J, Zhang L (2017a) Development of novel small InDel markers in jute (*Corchorus* spp.). Trop Plant Biol 10:169–176

Zhang L, Ma X, Zhang X, Xu Y, Ibrahim AK, Yao J, Huang H, Chen S, Liao Z, Zhang Q, Niyitanga S, Yu J, Liu Y, Xu X, Wang J, Tao A, Xu J, Chen S, Yang X, He Q, Lin L, Fang P, Zhang L, Ming R, Qi J, Zhang L (2021c) Reference genomes of the two cultivated jute species. Plant Biotechnol J 19:2235–2248. https://doi.org/10.1111/pbi.13652

Zhang L, Ming R., Zhang J, Tao A, Fang P, Qi J (2015) De novo transcriptome sequence and identification of major bast-related genes involved in cellulose biosynthesis in jute (*Corchorus capsularis* L.). BMC Genomics 16:1062. https://doi.org/10.1186/s12864-015-2256-z

Zhang L, Wan X, Xu Y, Niyitanga S, Qi J, Zhang L (2020) De novo assembly of transcriptome and genome-wide identification reveal GA_3 stress-responsive WRKY transcription factors involved in fiber formation in jute (*Corchorus capsularis*). BMC Plant Biol 20:403. https://doi.org/10.1186/s12870-020-02617-8

Zhang P, Lyu D, Jia L, He J, Qin S (2017b) Physiological and de novo transcriptome analysis of the fermentation mechanism of *Cerasus sachalinensis* roots in response to short-term waterlogging. BMC Genomics 18:649. https://doi.org/10.1186/s12864-017-4055-1

Zhao BT, Liang RQ, Ge LF, Li W, Xiao HS, Lin HX, Ruan KC, Jin YX (2007) Identification of drought-induced microRNAs in rice. Biochem Biophys Res Comm 354:585–590

Chapter 7
Genomic Designing for Abiotic Stress Resistance in Mulberry (*Morus* spp.)

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Abstract Mulberry is a fast growing deciduous, deep rooted, woody perennial plant that has been believed to have originated in sub-Himalayan tracts and spread into Africa, Asia, South America, Europe, North and South America. It is being cultivated widely across Asian countries for its leaves to feed the silk producing insect *Bombyx mori.* Besides leaf, other parts of mulberry such as fruits, timber, roots, etc. are also used for several economical purposes, which include human consumption and medical use. Although mulberry is moderately tolerant to major abiotic stresses such as drought and salt, the leaf yield and quality are affected by different types of stresses leading to expression of damages in different levels. To overcome these problems, efforts have been made to develop stress tolerant varieties through traditional breeding methods. However, as mulberry is highly heterozygous with a long juvenile period, the success rate of developing varieties with stress tolerance is quite meager. Since modern biotechnological tools have the potential to circumvent many of the constraints of traditional breeding, attempts have recently been made to employ biotechnological tools for mulberry crop improvement. The whole genome sequencing of mulberry has been done in two species and also transgenic mulberry with enhanced tolerance to drought and cold have been developed. Molecular markers have been used to assess the genetic diversity and mapping of genes. This chapter provides a detailed account of the efforts and achievements made through both traditional plant breeding and modern biotechnological techniques for the improvement of the mulberry to provide both leaf and fruits for the welfare of humanity.

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7.1 Introduction

Silk is the most elegant textile fiber in the world with unparallel grandeur, natural sheen, and inherent affinity for dyes, high absorbance, light weight, soft touch, and high durability. Thus, it is widely called as the "Queen of Textiles". It is a natural fiber with unique strength and elasticity. Properties like breaking strength of about 4 g/denier and elongation of about 20% make silk an excellent raw material for the textile industry (Baruah and Gogoi 2013). Although, there are several insects such as Silkworms, Raspy crickets, Honeybee, bumblebee, Bulldog ants, Weaver ants, Webspinners, Hornets, Silverfish, Mayflies, Thrips, Leaf hoppers, Beetles, Lacewings, Fleas, Flies, Midges, Caterpillars, Parasitic wasps that produce silk, the silk that is produced by a few insects like the tropical tasar silkworm (*Antheraea mylitta*), the oak-tasar silkworm (*A. proylei, A. roylei, A. pernyii, A. yamamai,* and *A. polyphemus*) the eri-silkworm (*Samia ricini*) and the muga silkworm (*A. assamensis*) is used widely for commercial purposes, especially in textile industries. Among the silks, the mulberry silk from *Bombyx mori* L. (Bombycidae) is the most prominent one as it covers more than 90% of the textile silk fiber. Since *Bombyx mori* L. is a monophagous insect that eats only the leaves of mulberry, due to certain volatile chemicals present in the leaf that activate the olfactory receptors in the antennae of silkworm larvae, mulberry occupies a key place in the sericulture industry. The silk industry starts with mulberry cultivation and moves through silkworm rearing to silk yarn production and fabric making. These activities generate huge employment opportunities for the rural and sub-urban populations, thereby playing a significant role in the development of the rural economy of many of the Asian countries. It is also to be noted that production of mulberry leaf alone covers more than 65% of the total expenditure for silkworm cocoon production (Lakshmanan 2007) making mulberry leaf production a vital component of sericulture in a country.

7.1.1 Mulberry and Its Economic Significance

Mulberry is a fast growing deciduous, deep rooted, woody perennial tree that has originated in the sub-Himalayan tracts and later spread into other regions such as Africa, Asia, South America, Europe, North and South America (Le Houerou 1980; Rodríguez et al. 1994). According to a legend, mulberry domestication was started in China during the Emperor Chin Shih Huang Ti in 2690 BC and slowly it spread to different parts of the world (Nanavaty 1990). Now, mulberry could be seen in all major continents including Asia, Europe, Africa, and America (Sanchez 2000a, b). Nearly 68 species were designated under the genus *Morus*, though only *M. alba* and its close species are cultivated widely for sericulture purposes (Katsumata 1979; Sanjappa

1989). Mulberry is reported to be moderately resistant to biotic and abiotic stresses, hence present in different terraints (Fig. 7.1), though for luxurious growth it needs flat land with deep, fertile, well drained, and loamy to clayey, porous soil with good moisture holding capacity and the pH ranging from 6.5 to 6.8. The mulberry leaves are simple, alternate, stipulate, petiolate, entire or lobed. The inflorescence is a catkin with pendent or drooping peduncle bearing unisexual flowers, mostly developed from axillary buds. Male catkins are usually longer than female catkins and the florets are loosely arranged. The female catkin is small and compact with florents having bifid feathery stigma. The ovary is one-celled. Pollination is anemophilous. The fruit is a sorosis and the color of it varies from white to lavender to black depending on the species and level of ripening (Fig. 7.2).

As stated above, the most important use of mulberry is to feed the monophagous silkworm *Bombyx mori* but it is also used for feeding animals as it is highly nutritious, palatable and digestible (Uribe and Sanchez 2001). The fruit of mulberry is a delicious berry full of vitamins, minerals, aminoacids, carotenoids, flavonoids, anthocyanins, resveratrol, zeaxanthin, litein and alpha and beta carotenes, apigenin, luteolin, quercetin, morin, caffeic acid, gallic acid, rutin, umbelliferone, chlorogenic acid cyanidin-3-O-β-D-glucopyranoside and kaempferol etc. (Table 7.1; Asano et al.

Fig. 7.1 Mulberry can be seen in different form depending on the cultural and training practices. **a** *M. alba* in low bush form, **b** *M. alba* in medium bush form, **c** *M. laevigata* in high bush form, **d** *M. laevigata* in tree form, **e** *M. alba* in high bush form, **f** *M. serrata* in tree form (adapted from Vijayan et al. 2018)

Fig. 7.2 Inter and intra specific variability in the size and colour of fruits in a few mulberry species. **a** *M. indica*, **b** *M. indica*, **c** *M. alba*, **d** *M. laevigata*, **e** *M. laevigata*, **f** *M. serrata* (adapted from Vijayan et al. 2018)

2001; Ercisli and Orhan 2007; Hassimotto et al. 2007; Singhal et al. 2009; Yang et al. 2010; Yigit et al. 2010; Arfan et al. 2012). It is found that fresh fruits of mulberry contain nearly 20 mg/100 g ascorbic acid (Chu et al. 2001). Mulberry fruit also contains essential fatty acids like palmitic, oleic and linoleic acids useful for cell membrane formation and functioning of the nervous system (Ercisli and Orhan 2007; Imran et al. 2010). Mulberry fruits have been used medicinally as a worming agent, as a remedy for dysentery, and as a laxative, hypoglycemic, expectorant, anthelmintic, odontalgic, and emetic (Chen et al. 2006; Kang et al. 2006). Hong et al. (2004) observed that mulberry fruit strengthens the antioxidative defense system and reduces damaging oxidative substances in the erythrocytes of diabetes. Mulberry fruit is consumed afresh, and as juice, jelly, sauce, cakes etc. (Bae and Suh 2007).

The barks of root and stem are rich sources of phenolic compounds such as maclurin, rutin, isoquercitrin, resveratrol and morin and are used as purgative, anthelmintic, and astringent purposes (Chang et al. 2011). Mulberry wood is very hardy and smooth and is used for manufacturing sports articles, turnery items, house buildings, agricultural implements, furniture, spokes, poles, shafts and bent parts of carriage and carts. Mulberry trees are grown for landscaping (Tipton 1994).

Table 7.1 Chemical composition of mulberry fruit (*M. indica*)

Chemical constituents	Quantity
Carbohydrates	7.8–9.0%
Protein	0.5–1.4%
Fatty acids (linoleic, stearic and oleic acids in seeds	0.3–0.5%
Free acid (mainly malic acid)	1.1–1.8%
Fiber	0.9–1.3%
Ash	0.8–1.0%
Moisture	85–88%
Calcium	0.17–0.39%
Potassium	1.00–1.49%
Magnesium	0.09–0.10%
Sodium	0.01–0.02%
Phosphorus	0.18–0.21%
Sulfur	0.05–0.06%
Iron	0.17–0.19%
Carotene	0.16–0.17%
Ascorbic acid	11.0–12.5 mg/100 g
Nicotinic acid	0.7–0.8 mg/100 g
Thiamine	7.0–9.0 μg/100 g
Riboflavin	165–179 μg/100 g

7.1.2 Effect of Stress on Yield and Quality

The major abiotic factors that limit the productivity of mulberry are drought and salinity, especially in semiarid and arid regions (Dorcus and Vivekanandan 1997). Globally, it is estimated that nearly 20% of agricultural land and 50% of crop land are affected by abiotic stress (Yokoi et al. 2002) thereby reducing the global agricultural production by 11.9–13.4%. In India, about 142 million ha of land are affected by drought (http://www.gisdevelopment.net) and about 7.3 million ha of arable land are affected by salinity and alkalinity (http://www.cssri.org) and the affected area is increasing daily due to intensive irrigation practices (Chen et al. 2002). Tolerance of a plant to stress is a measure of economic yield in the stressed environment. Mulberry being a fast growing plant requires a high input of nutrients and soil moisture, thus, any changes in these factors affect the plant adversely. The moisture stress is a major problem for mulberry cultivation in most of the regions and the plant experiences water stress at different growth stages due to inadequate precipitation and/or non-availability of irrigation water, especially during the dry months (Susheelamma et al. 1990). In mulberry, the leaf yield is directly or indirectly contributed by several characters that are governed by a wide array of physio-biochemical events (Misra et al. 2012). Moisture stress reduces leaf yield drastically under water stress (Susheelamma

et al. 1992; Rahman et al. 1999). For instance, the mulberry variety V1 in southern India yields 60 MT/ha/yr under irrigated conditions but under rainfed conditions it yields only 16–17 Mt/ha/yr. Likewise, S1635, another variety, yields 35 mt/ha/yr under irrigated conditions but only 14–17 MT/ha/yr under rainfed conditions. In another study, the effect of water stress on photosynthesis was determined in five mulberry cultivars (*M. alba* L. cv. K-2, MR-2, BC2-59, S-13 and TR-10) by imposing moisture stress ranging from 0.5 to 2.0 Mpa and it was found that photosynthetic rates and the activities of ribulose-1,5-bisphosphate carboxylase varied considerably among the genotypes under stress conditions Of the five mulberry cultivars, S-13 and $BC_2$59 showed higher photosynthetic rates, ribulose-1,5-bisphosphate carboxylase activity, high sucrose phosphate synthase activity and photochemical efficiency of PSII compared to the same by other varieties (Chaitanya et al. 2003). It was reported that leaf enlargement, stomatal conductance and photosynthetic activity are directly affected by leaf turgor potential (Ashraf et al. 2002). In order to maintain adequate water potential plants make osmotic adjustments through the accumulation of solutes to reduce the osmotic potential and to maintain turgor of plants (Ashraf 1994). It has also been seen that the chlorophyll formation was adversely affected by water stress leading to reduced photosynthetic activity and low dry matter production (Misra et al. 2012). The rate of transpiration was significantly reduced under severe water stress and it was observed that the degree of water stress in plants strongly impacts the rate of transpiration (Kramer 1983) and irrigation at seven day interval is essential for normal growth of mulberry (Misra et al. 2012). The higher leaf yield production in tolerant genotypes can be attributed to minimal plasticity in foliar gas exchange traits and better quantitative growth under low water regimes (Guha et al. 2010).

Another important stress that affects mulberry is soil salinity. It was observed that certain mulberry genotypes can sustain nearly 50% of its normal growth at a salinity imposed by 0.5% NaCl (EC 10.1 dS m^{-1}) (Vijayan et al. 2008a). Since many field crops such as date palm, olive, pomegranate and jujube can tolerate a salinity of EC 8–16 dS m^{-1} (Shannon and Grieve 1999; McKersie and Leshem 1994; Gucci et al. 1997), mulberry can be considered as a moderately tolerant species. The most common effects of salinity on mulberry are yellowing of younger leaves, appearance of puckered lesions between leaf veins and retardation of growth, early senescence, decreased photosynthesis, respiratory changes, loss of cellular integrity, tissue necrosis, and even death of the plant (Vijayan et al. 2008a). The growth and development of the plant was severely affected by salinity (Table 7.2). The leaf yield was decreased by 79.75% and 92.31% respectively under a salinity imposed by 0.75% and 1.00% NaCl in susceptible varieties and by 59.61 and 74.21% respectively in tolerant varieties (Table 7.3). However, the leaf pigments (Table 7.4) showed increase in low salinity, but reduction was observed in higher salinity (Table 7.3), and proline and Na^+ increased consistently with higher salinity (Table 7.5) (Kumar et al. 2003; Ramanjulu et al. 1993, 1994; Ramanjulu and Sudhakar 2000; Vijayan et al. 2008a). The protein content in the leaves was also seen decreasing under salinity in mulberry (Vijayan et al. 2007, 2008a, b).

Table 7.2 Effect of salinity on growth of mulberry (*Source* Vijayan et al. 2008a)

Genotype	Number of branches sprouted						Height of the plant (cm)					
	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean
E′ black	2.33	3.33	2.00	2.00	1.66	2.67	32.83	31.33	22.33	20.33	16.17	24.60
C776	5.66	3.00	4.00	4.00	3.33	4.06	36.67	38.00	30.00	30.00	17.00	30.33
Rotundiloba	5.00	5.33	4.00	3.33	2.33	4.00	31.00	29.00	28.00	26.67	25.00	28.11
Mandalya	4.00	3.00	3.33	2.66	2.00	3.13	37.00	28.67	29.67	17.33	6.00	23.13
Tollygunj	4.66	3.00	2.67	2.00	1.33	2.73	42.00	18.67	19.33	13.00	9.33	20.46
Mean	4.33	3.53	3.20	2.87	2.26		34.90	29.13	24.86	21.46	11.52	
F-values												
G (df 4,48)	11.21**						9.61**					
S (df 4,48)	10.7**						31.73**					
Gx S (df 16,48)	1.74						3.25**					

** = significance at $p < 0.01$; G = Genotype; S = Salt concentration: T1-0.00%, T2-0.25%, T3-0.50%, T4-0.75%, T5-1.00% NaCl

Table 7.3 Effect of salinity on leaf size and leaf yield of mulberry (*Source* Vijayan et al. 2008a)

Genotype	Leaf size (cm^2)						Leaf yield (gm)					
	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean
English black	50.39	44.49	44.57	36.46	22.17	39.62	4.07	3.00	2.17	1.43	0.89	2.31
C776	66.62	58.91	54.01	49.17	36.84	53.11	4.11	4.51	2.58	1.66	1.06	2.78
Rotundiloba	53.79	48.32	30.40	31.54	22.82	37.37	4.70	3.78	2.89	1.83	0.66	2.77
Mandalya	42.53	45.57	35.40	22.13	19.57	33.05	4.84	3.04	1.90	0.98	0.46	2.24
Tollygunj	32.37	32.30	37.24	23.19	15.52	28.12	3.74	1.86	1.13	0.84	0.28	1.57
Mean	49.14	45.89	40.33	32.50	23.38		4.29	3.24	2.13	1.35	0.67	
F-values												
G (df 4,48)	17.63**						54.48**					
S (df 4,48)	21.73**						74.01**					
Gx S (df 16,48)	0.835						9.16**					

** = significance at $p < 0.01$; G = Genotype; S = Salt concentration; T1-0.00%, T2-0.25%, T3-0.50%, T4-0.75%, T5-1.00% NaCl

Table 7.4 Effect of salinity on chlorophyll, protein in five mulberry genotypes under different salinity levels (*Source* Vijayan et al. 2008a)

Genotype	Mean Chlorophyll (mg/gm fr.wt)						Mean protein (mg/gm fr.wt)					
	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean
English black	2.31	2.36	2.56	1.69	1.56	2.10	26.17	21.64	21.32	25.17	20.86	23.03
C776	1.94	1.96	2.00	1.68	1.21	1.65	19.57	20.99	22.68	25.77	24.25	22.65
Rotundiloba	1.37	1.83	2.16	1.40	1.01	1.55	30.65	27.75	30.21	27.78	23.21	27.92
Mandalya	2.30	1.18	1.10	1.02	0.91	1.07	22.27	21.63	18.38	17.52	13.02	18.57
Tollygunj	1.81	1.06	1.31	1.31	0.98	1.28	21.99	19.50	17.43	17.53	12.84	17.85
Mean	1.72	1.56	1.83	1.42	1.13		24.13	22.30	22.00	22.75	18.83	
F-values												
G (df 4,48)	92.23**						85.72**					
S (df 4,48)	45.93**						19.89**					
Gx S (df 16,48)	8.09**						7.04**					

** = significance at $p < 0.01$; G = Genotype; S = Salt concentration; T1-0.00%, T2-0.25%, T3-0.50%, T4-0.75%, T5-1.00% NaCl

Table 7.5 Effect of salinity on sugar and proline content in mulberry (adapted from Vijayan et al. 2008a)

Genotype	Mean proline (μg/gm fr.wt)						Mean K: Na ratio					
	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean
English black	0.150	0.263	0.610	1.090	1.430	0.709	15.04	12.71	7.62	5.93	3.84	9.03
C776	0.130	0.190	0.263	0.270	0.960	0.363	12.64	14.20	7.78	8.02	6.74	9.88
Rotundiloba	0.087	0.117	0.180	0.260	0.380	0.205	15.99	8.41	5.49	3.63	2.16	7.08
Mandalya	0.057	0.067	0.080	0.123	0.273	0.120	10.49	9.17	6.26	3.09	1.87	6.18
Tollygunj	0.100	0.200	0.193	0.200	0.213	0.181	9.08	6.55	5.14	4.68	2.89	5.67
Mean	0.105	0.167	0.265	0.389	0.651		12.65	10.21	6.46	5.01	3.50	
F-values												
G (df 4,48)	173.40**						30.56**					
S (df 4,48)	143.96**						132.01**					
Gx S (df 16,48)	31.03**						5.27**					

** = significance at $p < 0.01$; G = Genotype; S = Salt concentration; T1-0.00%, T2-0.25%, T3-0.50%, T4-0.75%, T5-1.00% NaCl

The ideal temperature for optimal growth of mulberry ranges from 26 to 30 °C and the growth reduces significantly under low temperature. When the ambient temperature falls below 13 °C, the growth stops completely and the plant enters into a period of dormancy (Anonymous 1995a). Similarly, when the soil moisture falls below 60% of the field capacity the growth of mulberry becomes sluggish and if it falls below 25% it stops the growth altogether (Ohyama 1966). The CO_2 concentration in the atmosphere also impacts the growth and development as the plant height increases under elevated CO_2 conditions (Lavanya et al. 2017).

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

The ongoing scientific and technological developments and the consequent industrial and economic booms are forcing many countries to convert arable land into townships and industrial zones. This loss of arable is directly in conflict with the needs of the world population. Thus, farming has to be continued by finding alternative areas. A vast area of land is affected by salt and drought, which is projected to increase by 1.5 billion in the next 20 years. Since these types of stress affected lands are of special nature they require special technologies for cultivation of plants. Such special technological come with heavy costs. Thus, cost effective technologies are to be adopted. One of the most attractive and less expensive methods is to develop stress tolerant varieties (Agarwal et al. 1982). Further, mulberry being a cash crop, it has to compete with other crops for the marginal lands available for agriculture. To make things worse, climatic changes caused by global warming have also profoundly impacted mulberry cultivation. It is reported that global warming brings in extreme weather conditions such as drought, unseasonal rain, prolonged winter, emergence of new pest and disease etc. and affects plant growth and development (Jiang et al. 2016). Thus, it is necessary to develop mulberry varieties that can grow well in the marginal lands under varied climatic conditions.

7.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

To develop varieties genetic resources with adequate variability is a prerequisite. Keeping this in view, countries have collected, characterized and maintained a good amount of germplasm. In general, the mulberry germplasm includes traditional cultivars, elite and special genotypes developed by plant breeders and other researchers, wild relatives of the domesticated species, and wild species. Well established mulberry germplasm banks are available in Japan, China, India and many other countries where sericulture is one of the major avocations (Table 7.6). China

Table 7.6 Mulberry accessions in the germplasm banks of a few countries

Country	Mulberry accession
Japan	1375
China	3000
South Korea	208
India	1254
Bulgaria	140
Italy	50
France	70
Indonesia	5
Taiwan	5
Argentina	2
Colombia	4
Mexico	5
Peru	2
USA	23

keeps more than 2600 germplasm accessions, in provinces like Zhejiang, Jiangsu, Guangdong, Guangxi, Shandong, Sichun, Anhui, Hubei, Hunan, Hebei, Shanxi, Shuanxi and Xinjiang (Pan 2000). Japan has more than 1375 germplasm accessions (Machii et al. 1999) and India holds more than 1254 (Indigenous-984 and Exotic-270) mulberry accessions (Table 7.7). In Bulgaria, more than 140 mulberry accessions are maintained at SES-Vratza (Tzenov 2002) and Korea keeps 205 indigenous, 150 exotic and 259 unclassified strains.

Although continuous efforts have been made to develop varieties (Vijayan et al. 2009a), baring a few successes, not much progress could be achieved due to the complexity associated with the complex nature of the targeted traits, as most of them are contributed by several associated traits controlled by several genes (Namkoong et al. 1988; Vijayan et al. 2010). Further, to develop and release a mulberry variety it takes a minimum of 15 years (Fig. 7.3). Another major bottle neck for the development of mulberry varieties is the paucity of information on the genetic control of most of the agronomically important traits. Thus, it is difficult to transfer desirable traits from unadapted genotypes to elite lines through introgression and gene pyramiding as often desired traits are accompanied by unwanted traits, a phenomenon called linkage drag. The other issues associated with mulberry breeding are high heterozygosity of the plant as till date no inbred lines could be developed for breeding (Vijayan et al. 1997a, b). Thus, inshort, the major limitations of traditional breeding in mulberry are (1) understanding the basis of heterosis and prediction of hybrid performance, (2) identification of useful genetic factors in divergent populations or lines, (3) introgression of desired traits with minimal linkage drag and (4) understanding the genotype by environment interaction, remains unresolved. In order to resolve these problems, proper understandings on (a) the number of genetic factors (loci) influencing the expression of the traits, (b) the chromosomal location of these loci, (c) the relative

Table 7.7 Effect of urea and *Azatobactor chroococcum* on leaf yield of mulberry under different NaCl concentrations

Variety	NaCl	Branches (nos)				Height (cm)				Leaf yield/plant/(gm)			
		Urea	AS	AF	C	Urea	AS	AF	C	Urea	AS	AF	C
Mandalaya	0.00	3.33	6.00	5.33	5.00	49.67	52.00	51.00	47.00	17.80	24.65	26.57	12.25
	0.25	4.00	6.33	4.33	4.33	37.33	48.67	43.00	44.33	15.37	22.32	20.91	15.72
	0.50	3.00	2.67	3.33	2.67	48.33	39.33	36.67	26.33	10.32	13.81	12.90	6.12
	0.75	2.33	2.00	2.67	2.00	38.00	27.33	32.67	25.33	6.46	8.27	8.44	3.38
	1.00	1.67	1.67	2.33	1.33	27.33	18.00	19.33	12.67	3.80	3.93	4.03	1.74
	Mean	2.87^{a*}	3.73^{b}	3.60^{ab}	3.07^{ab}	40.13	37.07	36.53	31.13	10.75^{b}	14.59^{c}	14.57^{c}	7.84^{a}
C776	0.00	5.00	5.00	4.00	4.00	48.00	46.33	53.33	45.67	19.82	16.74	21.91	17.22
	0.25	5.00	3.67	3.67	3.00	45.00	43.67	43.00	39.33	16.85	18.42	17.77	20.74
	0.50	5.00	3.67	3.67	2.67	36.00	40.33	35.67	32.00	14.66	12.74	15.74	8.39
	0.75	3.00	2.33	2.33	2.33	28.00	35.00	30.33	31.33	11.61	8.51	9.75	5.89
	1.00	2.00	1.67	2.00	1.67	22.67	17.67	15.00	19.00	5.62	6.50	6.25	3.84
	Mean	4.00^{b}	3.27^{ab}	3.13^{ab}	2.73^{a}	35.93	36.60	35.47	33.47	13.65^{b}	12.58^{ab}	14.28^{b}	11.22^{a}
Rotundiloba	0.00	6.67	8.00	11.00	5.00	43.33	38.33	41.33	37.67	15.85	13.01	18.15	13.52
	0.25	5.33	7.00	7.00	4.00	43.67	31.33	37.67	34.33	12.90	9.54	15.05	2.61
	0.50	4.00	4.67	5.00	2.33	29.00	26.00	29.00	20.67	7.40	5.60	9.44	3.81
	0.75	2.33	3.3	4.33	2.00	22.67	24.33	27.33	18.33	4.96	3.99	7.54	3.14
	1.00	2.00	2.00	2.33	1.67	16.00	18.00	17.33	13.33	3.40	2.68	5.08	2.10
	Mean	4.07^{b}	5.00^{c}	5.93^{d}	3.00^{a}	30.93	27.60	30.50	25.07	8.90^{b}	6.96^{ab}	11.05^{c}	5.04^{a}
Tollygunj	0.00	4.00	5.67	5.67	3.67	45.33	48.00	57.33	43.33	3.68	13.26	17.12	7.19
	0.25	2.67	3.67	4.67	3.33	33.00	38.33	41.67	26.67	4.00	8.17	12.07	4.74

(continued)

Table 7.7 (continued)

Variety	NaCl	Branches (nos)				Height (cm)				Leaf yield/plant/(gm)			
		Urea	AS	AF	C	Urea	AS	AF	C	Urea	AS	AF	C
	0.50	2.33	2.67	3.67	2.67	21.67	29.33	37.67	26.33	1.88	3.76	6.02	2.48
	0.75	2.33	2.67	2.67	2.33	19.33	20.33	23.00	21.67	1.38	2.14	4.48	2.00
	1.00	1.33	1.33	1.67	1.67	8.67	13.00	19.67	13.33	0.84	1.67	3.14	0.66
	Mean	2.53^{a}	3.20^{ab}	3.67^{b}	2.73^{ab}	25.60	29.80	35.87	25.67	2.90^{a}	5.80^{b}	8.57^{c}	3.41^{ab}
Mean		3.37^{b}	3.80^{bc}	$4.13b^{c}$	2.88^{a}	32.79^{ab}	34.55^{ab}	36.07^{b}	30.20^{a}	9.80^{ab}	10.88^{ab}	13.04^{b}	7.49^{a}

* Values with same superscript letters are not significantly different at $P > 0.05$

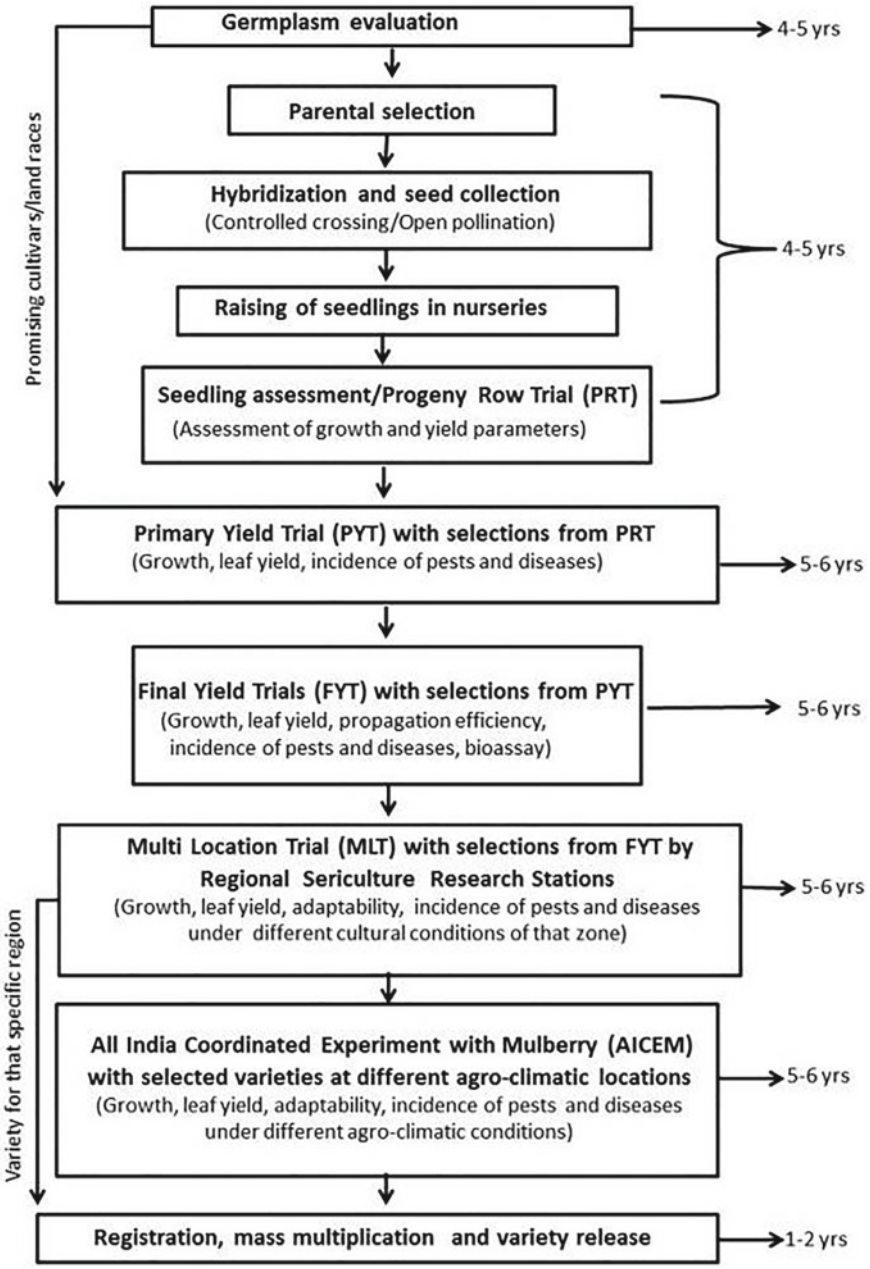

Fig. 7.3 Steps and time requirement for mulberry variety development

size of the contribution of individual loci to trait expression, (d) pleiotropic effects, (e) epistatic interactions among genetic factors, and (f) variation of expression of individual factors in different environments are required. To overcome these problems, it is essential to adopt the tools of genomics to make mulberry breeding, especially for the selection of suitable parents and hybrids, faster and reliable.

7.2 Description on Different Biotic Stresses

Owing to the sessile nature, plants are subjected to all changes in the surroundings, which include both abiotic stresses caused by heat, drought, cold, flood and salinity and biotic stresses caused by pests and pathogens. In order to survive and grow, plants have to adapt to the changing environmental conditions by developing different mechanisms. However, this adaptation often adversely affects growth, development and yields as much energy and resources need to be expended. Modern crop varieties are often more vulnerable to stress as they are developed mostly for higher yield. Since, stress in the form of drought and flood is becoming more common, it is imperative to develop varieties balancing both yield and adaptation. To develop such plant varieties, it is essential to understand the tolerance mechanisms in mulberry.

7.2.1 Root Characters

Mulberry is a deep rooted perennial tree that grows well in flat, deep, fertile, well drained, and loamy to clayey soil with pH ranging from 6.5 to 6.8. Mulberry roots are larger in diameter near the base and taper rapidly with the formation of secondary and tertiary roots and root hairs. Root hairs form fibrous mats to help plant uptake high concentrations of nutrients from the soil (Jungk 2001). Under stress conditions mulberry roots take up moisture through the cell membranes via osmosis, but with increase in the concentration of the solution outside the root zone, it becomes harder to absorb water from the soil, affecting the growth and yield. Under high salt concentration, water moves from the roots to the surrounding solution making the plant to lose moisture. Under a salinity imposed by 0.5% NaCl, the root growth was found reducing by 15.4–85.9% (Lu et al. 2017). It has also been found that stressed mulberry had significant changes in its root/shoot ratio (Liu et al. 2019).

7.2.2 Heat Tolerance

The ideal temperature for the luxurious growth of mulberry is 26–30 °C and any variation from that range affects the growth and development. From an experiment on activities of ribulose-1,5-bisphosphate carboxylase (RuBPC) and sucrose phosphate

synthase (SPS), photosystem 2 (PS 2) activity, chlorophyll (Chl), carotenoid (Car), starch, sucrose (Suc), amino acid, free proline, protein and nucleic acid contents in leaves under high temperature (40 °C) it is seen that high temperature markedly reduced the activities of RuBPC and SPS in leaf extracts, soluble protein, sugar metabolism and sucrose-starch balance (Chaitanya et al. 2001). The total amino acid content on the other hand increased along with the accumulation of proline in high temperature-stressed leaves (Chaitanya et al. 2001). It was also observed that the foliar nitrogen and nucleic acids (DNA and RNA) reduced considerably suggesting the high sensitivity of mulberry to high temperature.

7.2.3 Cold Tolerance

Cold affects mulberry considerably as the ambient temperature falls below 13 °C, the growth stops completely and the plant enters into a period of dormancy (Anonymous 1995a). However, there is a varietal difference in the response to severe cold. A few varieties from the temperate regions of Russia and China are known to have better tolerance ability than others (Pryluckyi 1969). Varietal differences was also observed in mulberry as a study with 57 mulberry germplasm accessions of five *Morus* spp., 12.08% leaf damage was noted in tolerant variety (S-146), 33.29% damage observed in susceptible variety C-763 (Ahanger et al. 2013). It was also noted that the tallest branch in a tree registered more damage (14.32%) than median (11.56%) and shortest branches (8.70%) (Ahanger et al. 2013). Shabnam et al. (2012) reported species difference towards cold tolerance by comparing five species of mulberry. *M. bombycis* exhibited greatest cold hardiness and resistance to die-back as compared to *M. alba*, *M. indica*, *M. multicaulis* and *M. kayayama*. It has also been observed that mulberry varieties exposed to severe cold evolve sdeveral mechanisms to enhance their freezing tolerance (Shukla et al. 2016). *Morus alba* and *M. indica* collections made from extreme cold climatic conditions of Ladakh, Lolab valley and Gurez regions of Jammu and Kashmir in India have shown resistance towards frost damage (ranging from 1.85 to 4.72%) as compared to 12% damages noticed in a commercially grown mulberry variety 'Goshoerami' (Shabnam et al. 2016, 2018). Shukla et al. (2018) performed gene expression analysis of these mulberry genotypes and found that Gurez genotype displayed significant up-regulation of WRKY46, spermidine synthase, ERD10, TIFY10, CPI, COR413 cold tolerant genes under both laboratory and field conditions compared to other mulberry genotypes. Although, the mechanism of cold tolerance is not yet deciphered clearly, it has been observed that during cold exposure, plants accumulate several gene products including high and low molecular weight cryoprotectants (Nanjo et al. 1999), Dehydrin proteins (Shukla et al. 2016). Similarly, Chen et al. (2018) observed that unsaturated fatty acids content and superoxide dismutase (SOD) activity were higher in tolerant varieties as compared to those in sensitive varieties. Under cold conditions, expression of mSOD1, mKCS1 and mAKR2A was reduced drastically in tolerant variety, whereas expression of mFADII increased suggesting the possible involvement of mAKR2A

in imparting tolerance in mulberry. Similarly, it has also been observed that different tissues in the same plant exhibits different tolerance level to cold as meristematic tissues have better tolerance than mature tissues (Sakai and Larcher 1987). Thus, the extent of the damage caused by low temperature depends on many factors, such as its developmental stage, the duration and severity of cold, the rates of cooling. Morphological differences were also noticed as the size of guard cells and the number of chloroplast in the leaf of higher cold hardy varieties than the same in cold susceptible varieties (Mc Murphy and Rayburn 1992). Shukla et al. (2019) analyzed the expression stability of eight candidate reference genes in mulberry under different abiotic stress treatments including drought, salt, heat and cold stresses and concluded that Ubiquitin is the most stable gene under cold stress conditions which can be used for quantitative real-time PCR gene expression analysis in Mulberry (*Morus alba* L.).

7.2.4 Drought Tolerance

Drought is defined as "a period of abnormally dry weather sufficiently prolonged for the lack of water to cause serious hydrologic imbalance in the affected area." (Huschke 1959) and it is considered as the most detrimental abiotic stress resulting from insufficient rainfall and/or altered precipitation patterns (Toker et al. 2007). The severity of drought depends on its timing, duration and intensity (Serraj and Sinclair 2002). If drought is caused by low rainfall, it may be often accompanied by relatively high temperature, which promotes evaporation and effects photosynthesis, thus intensifying the effects of drought further reducing the yield. It is anticipated that occurrence of drought in many regions will increase in the coming years due to the climate changes brought about by global warming (Reynolds and Ortiz 2010). Drought interferes with the physiological as well as biochemical functions of the plant as a result the plant shows reduced growth and development. It reduces the leaf size, stem extension and root proliferation, water-use efficiency, photosynthesis, and accumulates reactive oxygen species, leading to negative impact on antioxidant metabolism, and consequently cell peroxidation damage (Smirnoff 1993; Sarkar et al. 2016, 2019). In order to survive under the drought conditions, plants acquired a number of mechanisms, which include enhancement of water uptake, reduction of water loss through transpirational loss, accumulation of low-molecular-weight osmolytes, plant growth substances and antioxidants (Levitt 1972). In general plants use more than one mechanism at a time to resist drought.

Mulberry is a fast growing plants, hence, needs more water and nutrients (Guha et al. 2010). Therefore, availability of water is one of the most important deciding factors of the growth of mulberry. Baring a few reports on photosynthetic characteristics in mulberry during water stress (Chaitanya et al. 2003; Ramanjulu et al. 1998; Thimmanaik et al. 2002) not much information on mulberry growth and development under moisture stress conditions is available. A study conducted on four popular cultivars of *Morus indica* L. cvs.V-1, MR-2, S-36 and K-2 revealed that drought causes marked down-regulation of net photosynthetic rate (Pn), stomatal conductance (gs),

and transpiration rate (E) while there is an upward trend in water use efficiency (WUE) (Guha et al. 2009). Likewise, all four cultivars showed increase in carotenoids, ascorbic acid, glutathione, a-tocopherol and proline in the leaf. An endogenous loss of α-tocopherol and higher lipid peroxidation was also noticed under water deficit regimes (Guha et al. 2009). It is also observed that greater rooting vigour and leaf hydration status, minimal stomatal inhibition and stabilized photochemistry might play major roles in maintaining higher *Pn* and associated gas exchange functions in drought-tolerant mulberry genotypes under water stress conditions (Guha et al. 2010). To increase the drought tolerance in mulberry both traditional and modern biotechnological methods have to be employed.

7.2.5 Flooding and Submergence Tolerance

Flood, an overflow of water submerging areas for a considerable period, is considered as an abiotic stress to plants as imposes complex stresses on crop plants through depletion of oxygen in the soil (Voesenek and Bailey-Serres 2015). Thus, the major cause of stress during flood is the restriction of gas diffusion between the plant and its surroundings leading to limitation of heterotrophic energy production in mitochondria (Mustroph 2018). Flooding can be classified into two, waterlogging, where only the root system inside the soil is affected; and submergence, where part or the whole shoot of the plant is submerged in the water (Sasidharan et al. 2017). To tide over the stress caused by flood, plants develop several mechanisms such as anatomical and morphological modifications to improve gas exchange with the surroundings (Yamauchi et al. 2018). The formation of aerenchyma to improve gas transport and distribution inside submerged plant tissues is one of the major anatomical changes that take place in plants under flood (Colmer and Voesenek 2009). Some plant species may increase the shoot length rapidly to get their leaves out of the water or develop adventitious roots (Voesenek et al. 2004) and may make several structural and metabolic modifications such as cessation of growth, leaf abscission, down regulation of non-essential processes to further conserve energy and induction of anaerobic energy production (Colmer and Voesenek 2009; Van Dongen and Licausi 2015).

Although not much work has been done on mulberry to understand the flood tolerance, available information suggests that mulberry is moderately tolerant to flooding as it can withstand inundation up to a foot of water for a single growing season but succumbs to prolonged flood (Broadfoot and Williston 1973). Inundation of 12 mulberry varieties with varied tolerance level for 30 days up to a depth of 30 cm revealed that the tolerant varieties have thicker cortex and medullary rays. It is inferred that thicker cortex may protect the vascular bundles and the thicker medullary rays help radial translocation of solutes and thicker cork provides mechanical support to the plant. Further, the root hairs and branching of roots were reduced significantly (Ghosh et al. 2012). It was also noted that though in the tolerant variety the nitrate reductase activity declined sharply in 20–30 days of inundation, the nitrate reductase

activity resumed to normal in tolerant variety but in susceptible varieties it was not. Flooding also markedly reduced gibberellins levels in the xylem sap as the quantity of gibberellins production in root may have reduced significantly, resulting in depression of growth in waterlogged plants.

7.2.6 Nutrient Use Efficiency

Nutrient use efficiency (NUE) is an indicator of the efficiency of the plant to use the available mineral nutrients and produce the maximum. Israel and Rufty Junior (1988) defined nutritional efficiency as the relationship between total biomass and the amount of nutrient absorbed by plants. NUE is dependent on a number of factors such as nutrient absorption, influx kinetics, transportation, storage and assimilation It is also influenced by both physiological and genetic components of the plant and environmental factors including soil structure, texture, water holding capacity, waterlogging, salinity, alkalinity and drought and properties (Baligar et al. 2001). In other words NUE is the ability of the plant to increase the yield per unit of nutrient applied to soil. Thus, higher the NUE higher the yield and the profitability (Lopes and Guilherme 2000). NUE can be expressed and calculated by five different formats: (i) agronomic effectiveness (AE), (ii) physiological effectiveness (PE), (iii) grain yield efficiency (GYE), (iv) recovery efficiency (RE), and (v) utilization efficiency (UE) (Fageria 1992). NUE is considered as an essential factor deciding the growth and development of the plant under resource constraint soils as plant armed with efficient absorption and utilization of nutrients minimize the requirement of fertilizers, reduces the loss of nutrients. It is estimated that the overall efficiency of applied fertilizer could be about 50% for N, 10% for P, and 40% for K (Baligar and Bennett 1986). This clearly indicates the loss of applied fertilizers in the form of leaching, run-off, gaseous emission and fixation by soil. The absorption of nutrients is decided by the growth and development of roots, which in turn is greatly influenced by the physical structure of the soil, poor root growth leads to poor nutrient absorption. Besides soil evaluation and control, the plant should also be considered in terms of efficiency in nutrient utilization (Machado 1997). Cultivars have different nutrient absorbing capacities, which need to be considered before undertaking the cultivation. Thus, it is important to determine attainable yield for each cultivar and the recommendation of fertilizer dose should be determined accordingly as the amount of nutrients taken up by the cultivar is directly related with yield and profitability. Each cultivar has an attainable yield which determines the real nutrient demand and this information is essential to develop fertilizer recommendations (Espinosa and García 2009). In mulberry, the leaf is the main product and it is harvested repeatedly and pruned for sustaining the growth to get fresh leaf for silkworm rearing. This frequent harvesting and pruning are subjecting the plant to tremendous stress. To maintain the growth plants absorb huge quantity of nutrients from the soil. It has been reported that production of 20,000 kg mulberry-leaf (dry weight) removes 200–230 kg nitrogen, 40–45 kg phosphorus and 200–211 kg potash, besides, stems and older leaves also lock about 226 kg

nitrogen, 50 kg phosphorus and 204 kg potash (Rathore and Srinivasulu 2018). Thus, huge amount of fertilizers 20 MT FYM/ha/year and NPK @ 350:140:140/ha/year is applied in five split doses, each after 22–25 days of pruning. In case of East and North-Eastern zones, the NPK @336:180:112 kg/ha/yr is recommended. This application of huge quantity of chemical fertilizer is affecting the soil properties. To reduce the chemical fertilizer application biofertilizers have also been used and it is found that application of *Azatobacter* increases the leaf yield while sustain better soil structure (Sudhakar et al. 2000). It has also been shown that application of Arbuscular mycorrhizal symbiosis enhances the tolerance of mulberry to water deficit (Piao et al. 2016). Ahmed et al. (2017) demonstrated that the foliar application of fertilizer (LF) enhances leaf quantity and quality. Vijayan et al. (2007), demonstrated the beneficial effect of foliar application of *Azatobactor chroococcum* on mulberry leaf production under stress conditions like salinity (Table 7.7).

7.2.7 Water Use Efficiency

Water use efficiency (WUE) is a measure of the ability of the plant to convert water into plant biomass or grain (Briggs and Shantz 1913). In fact, it is being seen as the efficiency of the plant productivity and is measured as the ratio of the net photosynthetic rate (A_n) and transpiration rate (E). It is a cumulative effect of both genomic and environmental factors. Water use efficiency relies on number of genetic and environmental factors such as soil texture, structure, organic matter and beneficial microbial activity all contribute to the soil's ability to capture water and store it for use by plants, ability of the plant to absorb and assimilate the water to produce biomass, soil and atmospheric temperatures, precipitation, humidity and CO_2 concentration. Crop WUE is especially an important consideration where available water resources are limited or diminishing. Sinclair et al. (1984) listed five options for improving water-use efficiency relating to (1) biochemical alterations, (2) stomatal physiology, (3) alteration of the cropping environment, (4) improved harvest index, and (5) increased proportion of transpired water. It has also been found that fertilizer application can improve both the crop yield and WUE. The full potential of mulberry crop production is frequently decreased by the limitations of water and hence, it is a key factor for mulberry growth. Water stress caused a reduction in the size of the stomata accompanied by higher stomatal frequency. All the gas exchange parameters were severely affected by drought stress. In mulberry, it has been found that the water use efficiency (WUE) and levels of irrigation are inversely proportional i.e., higher the level of irrigation lower the WUE. Ananthakrishna et al. (1995) reported higher WUE in K2 mulberry in lower level of irrigation water applied and optimal WUE under 80% Epan value of irrigation under drip irrigation. Similarly, Benjamin et al. (1997) reported better WUE in mulberry under drip and sprinkler irrigation methods. A specially developed root structure at the University of Bangalore (UAS-B) is used for accurate and high throughput determination of water use efficiency and cellular level tolerance. A stable isotope based approach as a surrogate for WUE has been

standardised by Sheshshayee et al. (2003) for the assessment of genetic variability among mulberry accessions in relation to WUE. Nine contrasting mulberry genotypes for WUE and root traits were identified by screening about 300 germplasm for Δ13C Δ13C, growth and root parameters. This will pave the basis for the discovery of QTLs for those complex traits as well as to identify trait donor genotypes.

7.2.8 Salinity Tolerance

Salinity tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt such as sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+) and the anions: chloride (Cl^-), sulfate ($SO_4{}^{2-}$), bicarbonate ($HCO_3{}^-$), carbonate ($CO_3{}^{2-}$), and nitrate ($NO_3{}^-$). Hyper-saline soil water may also contain boron (B), selenium (Se), strontium (Sr), lithium (Li), silica (Si), rubidium (Rb), fluorine (F), molybdenum (Mo), manganese (Mn), barium (Ba), and aluminum (Al). The soil salinity can be divided into two categories, sodic and saline. Soil salinity is, generally, measured in units of electrical conductivity (dS m^{-1}) of a saturated soil paste extract (ECe). If the ECe is >4 dS m^{-1}, the exchangeable sodium percentage is <15% and pH < 8.5 the soil can be considered as saline (Szabolcs 1994). Salinity affects the growth and developments of mulberry in a variety of ways depending on the tolerance level, growth and developmental stages, physiological conditions, severity of the salt stress and influences of many other environmental factors such as relative humidity, temperature, radiation and air pollution (Shannon et al. 1994). The most common effects of salinity stress in mulberry are loss of turgor, growth reduction resulting in smaller leaves, shorter stature, early senescence, decreased photosynthesis, respiratory changes, loss of cellular integrity, tissue necrosis, and even death of the plant (Cheeseman 1988). The first visible symptom of salt injury in mulberry is the appearance of yellow patches in young leaves under low to moderate salinity (Vijayan et al. 2008a). The yellowing of leaf may be due to degradation of chlorophyll by the increased activity of chlorophyllase (Singh and Singh 1999). Under higher salinity burnt like lesions appeared in the leaves (Vijayan et al. 2008a). Early senescence of older leaves and retardation of growth followed under higher salinity as the salt promotes senescence of leaves by increasing the production of abscisic acid (ABA) and ethylene (Kefu et al. 1991; Zhao et al. 1992). In mulberry, an increase in leaf thickness was observed in response to salinity and it was the result of an increase in number of spongy layers rather than an increase in the size of palisade cells (Vijayan et al. 2008a). Salinity affetcs the rate of photosynthesis and stomatal conductance (g_s) (Golombek and Lüdders 1993; Tattini et al. 1995; Kumar et al. 1999), besides injuring the cell membrane leading to increased solute leakage (Hautala et al. 1992). It was seen that the salinity induced cell membrane damage was greatly influenced by the tolerance level of the genotypes (Vijayan et al. 2002), though the membrane leakage was reduced under higher $Ca_2{}^+$ (Leopold and Willing 1984). Proline, glycine betaine, and reactive oxygen species (ROS) such as superoxide radicals ($O_2{}^-$), Hydrogen

peroxide (H_2O_2) and hydroxyl radicals (OH*) are found increasing under saline stress (Ramanjulu and Sudhakar 2000; Vijayan et al. 2008a). Although certain mulberry genotypes were found sustaining nearly 50% of its normal growth at a salinity of 0.5% NaCl (EC 10.1 dS m^{-1}), most of the mulberry genotypes show detrimental effects at this level of salinity. Salinity also affected plant anatomy as leaves of plants growing in saline soils became thicker and more succulent as compared to that of the plants growing in non-saline soils (Shannon et al. 1994; Vijayan et al. 2008a). This increased leaf thickness may be part of the salt tolerant mechanisms as the leaf succulence may increase the CO_2 absorption per unit of leaf area by increasing the internal surface area per unit of leaf surface (Shannon et al. 1994). Photosynthesis was also affected severely (Kumar et al. 1999; Lakshmi et al. 1996) due to reduced stomatal conductance (g_s) and reduction in the pigment concentrations. Salinity also injured cell membranes and increased solute leakage (Vijayan et al. 2002).

7.2.9 Traditional Breeding Methods

Traditionally, mulberry is developed through hybridization and selection. The whole breeding process in mulberry starts with the characterization of germplasm accessions. Morphological, biochemical and physiological characters such as plant growth and development, rooting ability of the stem cutting, leaf yield, leaf moisture, protein and sugar contents, photosynthetic efficiency, physiological water use efficiency etc., have been used to group the accessions based on similarity in characters using different statistical methods. Parents of desired characters are selected and controlled hybridization is effected. Ripened fruits from controlled hybridization as well as those formed by natural hybridization in selected mother plants are collected to extract seeds. Seeds are sown in nursery beds. Screening and selection of hybrids, initially based on a few important traits like growth; branching, leaf texture, and disease susceptibility are done in progeny row trials (PRT). Since almost all mulberry accessions are highly heterozygous, and have long gestation period, traditional breeding methodologies mostly relied on the production of F1 hybrids (Sastry 1984). Hybrids with desirable traits, identified through the progeny row trial, are further evaluated in primary yield trail (PYT) for important agronomic, biochemical and silkworm feeding qualities. From the PYT, the top 5–10% hybrids are selected for detailed assessment in final yield trail using 3–5 replication and 25–49 plants per replication. Here, the plants are put to thorough assessment for leaf yield, leaf quality, adaptation, susceptibility to pest and diseases, rooting ability, response to agronomic practices, and bioassay through silkworm feeding. Once a hybrid is found to have almost all the desired traits, it is selected and mass multiplied vegetatively and further tested at different regions with the serizone (MLT). Usually, 8–9 hybrids are used for regional multi-location studies. Those hybrids which perform consistently well in all the seasons, locations and years are selected and further tested in All India Coordinated Experimental Trail (AICEM) to evaluate their performance in different agro-climatic conditions in India for a minimum of four years. The best performers

under the AICEM are released for commercial exploitation. Special techniques to develop triploids have also been developed as triploids in mulberry are known to have several advantages over their diploid counterparts (Vijayan and Chakraborti 1998). Thus, attempts have been made to develop triploids from desirable diploids via the development of tetraploids through the application of colchicine (Chakraborti et al. 1998) and crossing the colchicine induced tetraploids with diploids. Some of the popular mulberry varieties like Tr-10, Tr-23, S1635 are triploids developed as described above. These triploid varieties have wider adaptability and better tolerance to abiotic stresses.

7.2.10 Use of Morphological Markers

Plenty of research has been done on characterization of parental materials, screening of hybrids and evaluation of selected genotypes using morphological markers such as leaf shape and size, lenticels frequency, stem colour, flower color, stigma length and nature, fruit shape and colour, seed colour and size, plant height, stem length, number of branches, leaf retention capacity, moisture content and retention capacity, nodal length, leaf yield, biomass production, etc. (Bindroo et al. 1990; Sahu et al. 1995; Tikader et al. 2009; Vijayan et al. 1997a) along with others traits like adaptability to different climatic conditions, resistance to pests and diseases, tolerance to abiotic stresses like drought, salinity and cold, higher vegetative propagation ability, better leaf quality, and better coppicing ability (Vijayan et al. 2009a). However, the high heterozygosity, long juvenile period of the plant, multigenic and multifactorial nature of the characters, these morphological markers were found little use as they are highly varied across different climatic conditions and stages of growth (Vijayan et al. 2006a). Thus, relying exclusively on morphological traits may lead to misleading results.

7.2.11 Limitations and Prospect of Genomic Designing

In order to select appropriate parents for breeding, it is essential to have thorough knowledge of the total genetic makeup of the plant and the gene pool available at the disposal of the breeder. Traditionally, phenotypic characterization was employed to estimate the genetic diversity among germplasm accessions but due to the above stated limitations, it is essential to find more robust, environmentally stable, reproducible, easy to define, unbiased, ubiquitous markers for employing in the crop improvement programs of mulberry (Vijayan and Chatterjee 2003). As in other crops, it is possible to modify mulberry genetically with modern molecular tools. Genetic engineering through transgenesis and gene editing is much needed in mulberry to develop new varieties with desired traits.

7.3 Genetic Resources of Resistance/Tolerance Genes

The complete alleles present in a population are called gene pool. The conservation strategies used for mulberry like other vegetatively propagated plants encompass a wide spectrum of activities ranging from establishment of protected areas to building of DNA libraries (Tikader et al. 2009). If the genetic resources are conserved at the natural habitat itself, it is called in situ conservation (Tikader et al. 2009). Since the native habitat is much suitable for growth and development, it is rich in genetic diversity, thus, in situ conservation is considered the best strategy for conserving the whole genetic diversity of plant. In situ conservation offers several advantages such as the plant population under in situ conservation is open to free genetic exchange through random matings and recombinations, selectively advantageous mutations may accumulate and spread among the populations, and healthy competition among the members of the population help better adaptability and possession of greater genetic diversity. Thus, this permanent habitat preservation is a dynamic and evolutionarily potential way of plant genetic conservation (Frankel and Soule 1981). Conservation of genetic resources away from their natural habitat, in botanical gardens, research institutes, experiment stations, nurseries or home gardens and seed gene banks and is called ex situ conservation Ex situ filed gene banks of mulberry are developed through planting of stem cuttings/saplings or by grafting the buds on appropriate rootstocks. Mulberry genetic resources maintained in the ex situ germplasm of major sericultural important countries (Akio et al. 2002; Tzenov 2002; Pan 2003; Tikader and Dandin 2006). Mulberry genetic resources are also preserved through cryopreservation as it has proven to be a potentially ideal method for long-term preservation of mulberry due to the less requirement of space, labor, and cost. Under cryopreservation, the plant materials are stored at −196 °C in the liquid nitrogen. DNA banks can also be used for preserving the genetic information of mulberry.

7.3.1 Primary Gene Pool

The primary gene pool (GP-1) is the gene reservoir that is utilized easily for crop improvement as the crossing among accessions of primary gene pool is easy and it produces fertile offsprings. The primary gene can further divided into cultivated gene pool encompassing commercial stocks and landraces, and the wild gene pool comprising putative ancestors and closely related species that show a fair degree of fertile relationships with the domesticate ones (Allem et al. 2001). Thus, the primary gene pool of mulberry consists of primitive cultivars, natural hybrids, wild and weedy relatives, obsolete varieties, elite lines, breeding lines. A number of mulberry accessions have been collected, characterized and preserved by most of the sericulturally important countries (Table 7.8). This primary gene pool of mulberry has been evaluated for stress related genes and found identified several accessions with stress tolerance. In vitro screening of axillary buds and shoot tips was used to screen 10 mulberry

Table 7.8 Composition of the mulberry germplasm in India

Name of country	Country code	Accessions	Frequency (%)
Afghanistan	AFG	2	0.16
Australia	AST	2	0.16
Bangladesh	BGD	5	0.40
China	CHI	53	4.23
Cyprus	CYP	1	0.08
Egypt	EGY	3	0.24
France	FRA	32	2.55
Hungary	HUN	1	0.08
India	IND	984	78.5
Indonesia	IDA	6	0.48
Italy	ITA	7	0.56
Japan	JPA	70	5.58
Myanmar	MYN	7	0.56
Pakistan	PAK	8	0.64
Papua New Guinea	PAP	1	0.08
Paraguay	PAR	4	0.32
Philippines	PHI	1	0.08
Portugal	PRT	1	0.08
Russia	RUS	1	0.08
South Korea	SKR	6	0.48
Spain	ESP	2	0.16
Thailand	THI	11	0.88
Turkey	TUR	1	0.08
USA	USA	2	0.16
Venezuela	VEN	1	0.08
Vietnam	VTM	3	0.24
Zimbabwe	ZIM	11	0.88
Unidentified	–	28	2.23
Total		**1254**	**100.0**

genotypes (Hossain et al. 1991), screened 63 mulberry genotypes (Vijayan et al. 2003) and identified several salt tolerant accessions. Tewary et al. (2000) screened mulberry genotypes for osmotic stress tolerance by subjecting to stress caused by 1.0–10% polyethylene glycol (PEG). Likewise, Jhansi Lakshmi et al. (2014) screened 121 accessions for different physiological parameters that are associated with WUE (Water Use Efficiency) and drought tolerance and identified 16 accessions with better tolerance to drought stress.

7.3.2 Secondary Gene Pool

Although the species differentiation in mulberry is very thin and natural cross hybridization is very common among most of the cultivating species, there are a few species which show very poor hybridization with these domesticated species. Prominent among them are *M. serrata* Roxb, *M. cathayana* Hemsl., *M. laevigata* Wall., *M. wittiorum* Hand-Mazz., *M. nigra* Linn., and *M. mongolica* Schneid., and these species are considered to be the secondary gene (GP-2) pool of mulberry, even though these species cross with other species but produce less number of seeds to produce sterile hybrids as most of them are polyploids (Weiguo et al. 2007).

7.3.3 Tertiary Gene Pool

The tertiary gene pool (GP-3) consists of distantly related species of the primary gene pool and the crossing between these two is difficult and gets only sterile hybrids. Paper mulberry (*Broussonetia papyrifera*) may be one of the genuses which could be considered as the tertiary gene pool of mulberry as it belongs to the family Moraceae and has variable-shaped leaves that are rough to touch, the plant looks like a hybrid originated from a cross between mulberry and osage-orange.

7.3.4 Artificially Induced/Incorporated Traits/Genes

In order to expand the genetic base of mulberry, efforts have been made to generate variations through various means such as plant tissue culture, induced mutation, polyploidization, and genetic engineering. Through, induced mutations a few popular mulberry varieties such as S13, S34, S36 with enhanced drought tolerance were developed in India (Sastry et al. 1974). Induction of mutations with EMS in the genotype RFS135 resulted in isolation of varieties with wide economic values in sericulture (Anil Kumar et al. 2012, 2013); it was found that 0.1 and 0.3% EMS treatment were effective in altering the morphometric characters, biomass yield and phytochemical constituents such as proteins, reducing sugars, minerals and moisture content. Similarly, a somaclonal variant (SV1) was developed from a popular variety S1 (*M. alba*) and it showed increased branching, tolerance to drought and higher leaf yield potential (Chakraborti et al. 1999). Similarly, Susheelamma et al. (1996) isolated another somaclonal variant from plantlets developed through callus culture of var. S-14. The somaclonal variant showed beneficial traits like shorter internodal distance, thicker leaves, higher chlorophyll content, higher moisture content and better moisture retention capacity. New plant varieties have also been developed though induction of polyploidy. For instance, more than 30 tetraploids have been developed in India through colchicines treatment of diplid varieties and using these

tetraploids, triploid varieties with higher stress tolerance such as Tr-10, Tr-23 and S1635. Genetic engineering has also been employed to develop varieties by inserting genes of interest such as glycine gene *AlaBlb*, oryzacystatin gene *OC* (Wang et al. 2003), and the barley *HVA1* gene (Lal et al. 2008). The transgenic mulberry over-expressing *HVA1* showed increased cell membrane stability, higher relative water use efficiency and growth under salt stress (200 mM NaCl) in mulberry (Lal et al. 2008). The transgenic mulberry with tobacco osmotin gene showed higher tolerance to drought stress as osmotin and osmotin-like proteins are stress proteins belonging to the plant PR-5 group of proteins induced in several plant species in response to various types of biotic and abiotic stresses (Das et al. 2011). Further, attempts have also been made to develop seedless mulberry fruits. Dajiu is a single strain selected by the Guangdong hybrid mulberry offspring in Dalilang Village, Xiqiao District, Nanhai County, Guangdong Province in 1977. The No. 10 strain was named "Big 10".

7.4 Glimpses on Classical Genetics and Traditional Breeding

7.4.1 Classical Mapping Efforts

The genetics of mulberry remains a mystery as mulberry is highly heterozygous and due to various reasons including long juvenile period, inbreeding depression, non availability of inbred lines. Classical genetic maps are based on the sequential allocation of loci to a relative position on a chromosome based on the frequency of chromosome crossovers occurring during meiosis and not on their physical distance or location on the chromosome. With the advancement of modern molecular tools, linkage mapping is done using molecular markers. In mulberry, no genetic map has been developed through classical genetics.

7.4.2 Limitations of Classical Endeavors and Utility of Molecular Mapping

As stated elsewhere mulberry is a highly heterozygous perennial tree and most of the economically important traits are under the influence of a number of genes. The expression of most of these traits is, thus, highly influenced by environmental factors and stages of developments. A certain set of characters would appear under a given set of climatic conditions in a particular stage of development and others will appear in other set of conditions. Thus, the genetic base of all the characters of mulberry could not be elucidated with the morphological markers. Molecular markers, on the

other hand, are present in abundance, stable across the growing conditions and developmental stages, and are devoid of pleaotripic and epistatic effects. Thus, molecular markers were found much better than the phenotypic markers in assessing the genetic diversity, identification of parents and evaluation and selection of hybrids. Depending on the techniques used, these markers can be broadly classified as hybridization based markers and polymerase chain reaction (PCR) based markers. In hybridization based marker systems like restriction fragment polymorphism (RFLP), the DNA profiles are visualized by hybridizing the restriction enzyme-digested DNA blotted onto a solid membrane with radio labeled probe. In PCR based marker system, in vitro amplification of particular DNA sequences is carried out with the help of specifically or arbitrarily chosen primers and a thermostable enzyme, called *Taq* polymerase. The amplified fragments are separated electrophorectically on polyacrylamide gels or agarose gels and the banding patterns are detected by either staining or by autoradiography. Some of the important PCR based marker systems are random amplified polymorphic DNA (RAPD), amplified fragment polymorphism (AFLP), inter simple sequence repeats (ISSR), simple sequence repeats (SSR), expressed sequence tag (EST). Each of this marker system has its own merits and demerits. Markers like RFLP, SSR and EST are co-dominant in nature, thus, can detect genetic variability at allelic level. However, the development and utilization of these marker systems are costly, laborious and time taking. Thus, in mulberry, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Inter Simple Sequence Repeat markers were used for genetic divergence and molecular characterization of germplasm (Vijayan et al. 2005, 2006a; Huang et al. 2009; Krishnan et al. 2013). However, all the above stated markers had a number of inherent limitations including, dominant nature, reproducibility, anonymity etc. The advent of next generation sequencing and the drastic reduction in the cost of sequencing have enabled designing and utilization of more robust, reproducible and informative molecular markers such as SSR and SNPs. Using SSR markers, a number of QTL maps have been developed in mulberry for different traits (Sarkar et al. 2017). With the advent of NGS, efforts are underway to develop SNP panels for their utilization in mulberry.

7.4.3 Breeding Objectives: Positive and Negative Selection

Since the leaf is the primary product of mulberry in most Asian countries, the breeding was aimed at developing varieties with wider adaptive and higher leaf yield potential. Mulberry growth and leaf production depend on a number of factors and associated traits, thus, the breeding process always relies on certain markers/traits which contribute considerably to the growth and development of the plant. The selection process may be two types, positive and negative. The selection based on traits or markers that confer a selective advantage for the plant is called positive selection while those confer a disadvantage is called negative selection. Positive selection is

also called Darwinian selection and in which genes/traits/variants that have a selective advantage increase in number until they fix in the relevant population. On the other hand, negative selection, also called purifying selection, is a purging process wherein disadvantageous or deleterious alleles/genes/traits get eliminated from the population. The strength of selection varies between locus/genes. In the case of strong negative selection on a locus, the purging of deleterious variants will result in the occasional removal of linked variation, producing a decrease in the level of variation surrounding the locus under selection. In mulberry a number of traits have been identified which have a strong and positive correlation with the survival and leaf yield of mulberry under stress conditions and also a set of characters that have negative correlations (Vijayan et al. 2010). It has been found that the character association changes with the intensity of the stress imparted by salinity. Under normal condition, leaf yield is significantly and positively correlated with leaf size, root length, shoot length, protein content of the leaf and the photosynthesis of the plant. However, under a stress caused by 1.00% NaCl (ECe 19 dS m^{-1}) the leaf yield has highly significant correlation with plant height, leaf size, shoot weight, root weight, root length, protein, NRase activity and WUE of the plant. Likewise under normal cultural conditions the leaf weight and leaf moisture content showed a non-significant negative correlation with leaf yield. Thus, the selection strategy varies with traits and growing conditions.

7.4.4 Classical Breeding Achievements

Through classical breeding, a number of mulberry varieties have been developed by different countries. For instance, India has developed 27 mulberry varieties (Table 7.9), and China has developed 31 mulberry varieties (Table 7.10). The leaf productivity of these varieties increased considerably from 8 to 10 MT/ha/yr in traditional varieties to 60–65 MT/ha/year in the newly developed varieties. Similarly, a few varieties with stress resistance have been developed. AR-12 and C776 are examples of it.

7.4.5 Limitations of Traditional Breeding and Rationale for Molecular Breeding

The biggest limitation of traditional mulberry breeding is the long time required for developing a variety. It takes almost 15–20 years to develop a variety as mulberry has a long juvenile period and takes a minimum of 2–3 years to get the plant ready for developing the next generation. Further, lack of inbreds and the high heterozygosity associated with the accessions makes the genetic improvement through conventional breeding highly laborious. Hence, in most of the breeding plan, crosses between selected parents are made to develop F1 hybrids and the F1 hybrid is screened to select

Table 7.9 List of mulberry varieties being commercially exploited in India

Sl. No.	Variety	Breeding method	Area of cultivation
1	G4	Hybrid from *M. multicaulis* x S-13	South India Irrigated
2	G2	*M. multicaulis* x S34	Young age silkworm rearing in irrigated conditions
3	C2038	Hybrid from a cross between CF1 × C763	Eastern and NE India Irrigated
4	Tr23	Hybrid from a cross between T20 × S-162	Hills of Eastern India
5	Victory-1	Hybrid from a cross between S30 × C776	South India Irrigated
6	Vishala	Clonal selection	All India Irrigated condition
7	Anantha	Clonal selection	South India Irrigated
8	DD	Clonal selection	South India Irrigated
9	S-13	Selection from polycross (mixed pollen) progeny	South India Rainfed
10	S-34	Selection from polycross (mixed pollen) progeny	South India Rainfed
11	S-1	Introduction from (Mandalaya, Myanmar)	Eastern and NE India Irrigated
12	S-7999	Selection from open pollinated hybrids	Eastern and NE India Irrigated
13	S-1635	Triploid selection	Eastern and NE India Irrigated
14	S-36	Mutation breeding through EMS treatment of Berhampore Local	South India Irrigated
15	S-54	Mutation breeding through EMS treatment of Berhampore Local	South India Irrigated
16	Sahana	Kanva-2 × Kosen	Coconut shades of South India
17	RC-1	Punjab local × Kosen	Resource Constraint areas of South India
18	RC-2	Punjab local × Kosen	Resource Constraint areas of South India
19	AR-12	S41 (4x) × Ber. C776	Alkaline affected areas of Southern India
20	C776	Hybrid from a cross between English black and *C. multiculis*	Saline soils
21	C-1730	Hybrid from a cross between T-25 × S-162	Rain fed areas
22	C-2028	Hybrid from a cross between China White × S-1532	Water logged areas

(continued)

Table 7.9 (continued)

Sl. No.	Variety	Breeding method	Area of cultivation
23	S-146	Selection from open pollinated hybrids	N. India and Hills of J and K Irrigated
24	Tr-10	Triploid developed from "S1"	Hills of Eastern India
25	$BC_2$59	Back crossing of hybrid of Matigare local × Kosen with Kosen twice	Hills of Eastern India
26	Chak Majra	Selection from natural variability	Sub-temperate
27	China White	Clonal selection	Temperate

promising ones to put into next level of evaluation for variety development. Thus, trait specific improvement does not have much scope in this type of breeding programme as recurrent hybridization and selection take decades to be completed. Additionally, stress tolerance in plants is a complex phenomenon involving morphological and developmental changes as well as physiological and biochemical processes (Gill and Tuteja 2010; Hirayama and Shinozaki 2010). Screening for stress tolerance in the field is not an appropriate method as the levels of stress imposed by both salt and drought in the field can vary depending on season and soil depth. Plants also interact with many other environmental factors which interfere with the expression of stress tolerance. Therefore, plants have to be screened under controlled environmental conditions to facilitate true expression of their innate ability to tolerate salt and drought stresses. However, screening of a large number of F1 hybrids for stress tolerance by imparting stress is near prohibitive. Therefore, it is highly desirable to use molecular biological tools such as Marker Assisted selection and genetic modification through genetic engineering.

7.5 Brief on Diversity Analysis

7.5.1 Phenotype-Based Diversity Analysis

Evaluation of germplasm and grouping of the accessions based on diversity among them is required for selecting suitable parents for breeding. In the earlier days in the absence of molecular markers, germplasm was evaluated using phenotypic traits such as morphological traits and agronomical traits such as tolerance to drought, saline and alkaline stresses, early sprouting or winter hardiness, resistance to pests and diseases (Tikader and Kamble 2007). Tikader et al. (1995) explored the variability in the expression of sex in mulberry in 301 genotypes from diverse geographical origins, and found that nearly 16% was male, 53% female, 17% monoecious and 13% were bisexual. Significant variation was noted in the flowering time, anthesis

Table 7.10 List of high yielding mulberry varieties developed in China (*Source* Vijayan et al. 2011a)

Sl. No.	Variety	Selection and breeding method	Suitable zone
1	Xiansang 305	Mutation breeding	The Huanghe River valley
2	Beisangyihao	Selected from local seedling mulberry	The Changjiang River valley, The middle and lower reaches of the Huanghe River
3	Nongsang 8	Hybridization breeding	The Changjiang River valley
4	Huangluxuan	Selection from local variety	The Huanghe River valley
5	Jihu 4	Hybridization breeding	Northeast zone
6	Dazhonghua	Polyploidy breeding	The Changjiang River valley
7	Xinyiyuan	Mutation breeding	The Changjiang River valley, The middle and lower reaches of the Huanghe River
8	Nongsang 14	Hybridization breeding	The Changjiang River valley
9	Yu 237	Hybridization breeding	The Changjiang River valley
10	Xuanqiu 1	Selected from local seedling mulberry	Northeast zone
11	7307	Selected from local seedling mulberry	The Changjiang River valley
12	Husang 32	Selection from local variety	The Changjiang River valley, The middle and lower reaches of the Huanghe River
13	Xiang 7920	Hybridization breeding	The Changjiang River valley
14	Canzhuan 4	Selected from local seedling mulberry	The Changjiang River valley
15	Huamingsang	Selected from local seedling mulberry	ChizhouXuanzhouAnqing in Anhui province and Linyi in Shandong province
16	7946	Hybridization breeding	The Huanghe River valley
17	Yu 2	Hybridization breeding	The Changjiang River valley
18	Shigu 11-6	Mutation breeding	The Changjiang River valley, The middle and lower reaches of the Huanghe River
19	Xuan 792	Selection breeding	The Huanghe River valley
20	Yu 711	Hybridization breeding	The Changjiang River valley, The middle and lower reaches of the Huanghe River
21	Yu 151	Hybridization breeding	The Changjiang River valley
22	Hongxin 5	Hybridization breeding	The Changjiang River valley, The middle and lower reaches of the Huanghe River

(continued)

Table 7.10 (continued)

Sl. No.	Variety	Selection and breeding method	Suitable zone
23	Lunjiao 40	Selection from local variety	The Zhujiang River valley
24	Wan 7707	Selected from local seedling mulberry	Chizhou, Xuanzhou, Anqing in Anhui province and Linyi in Shandong province
25	Huangsang 14	Selected from local seedling mulberry	The Changjiang River valley
26	Lunjiao 40	Selection from local variety	The Zhujiang River valley
27	Shi 11	Selection from local variety	The Zhujiang River valley
28	Xinyizhilan	Introduced variety	The Changjiang River valley; The Huanghe River valley
29	Jialing 16	Polyploidy breeding	The Changjiang River valley
30	Tang10 × Lun 109	Hybrid mulberry seed	The Zhujiang River valley
31	Nongsang 12	Hybridization breeding	The Changjiang River valley

and floral characteristics. Variability was also observed in pollen grain viability, fruit morphology (Fig. 7.4) and seed setting %. Vijayan et al. (1999) estimated the genetic diversity among the 62 mulberry accessions indigenous to India, irrespective of their species and ploidy status. Significant genetic divergence was observed among these indigenous mulberry accessions on the basis of the leaf yield traits. Tikader and Kamble (2008) evaluated the genetic diversity of 50 mulberry germplasm accessions using eight agronomically important traits and found a high amount of genetic diversity among the accessions. Banerjee et al. (2011) evaluated the genetic diversity of twenty-five Indian mulberry accessions representing five species using 22 morphometric traits. Significant differences among germplasm accessions were observed for all 22 traits. Six principal components were identified, which explained >88% of the total variation. Though 18 major variables were included in the principal components, longest shoot length, leaf area, internodal distance, green and dry leaf weight, lamina length, lamina weight, root volume, and fresh and dry root weight were recognized as important variables. Chang et al. (2014) evaluated the genetic diversity of 27 mulberry accessions from seven *Morus* spp., using 20 vegetative traits, chilling requirement, and reproductive traits. Based on the study, a classification system was suggested with three clusters: (1) *M. laevigata*; (2) *M. atropurpurea*, *Morus bombycis*, *Morus australis* and *M. formosensis*; (3) *M. alba* and *M. latifolia*. The study also showed that *M. atropurpurea*, often regarded as a member of *M. alba*, is closed to *M. bombycis*, *M. australis* and *M. formosensis*. Peris et al. (2014) assessed the genetic diversity among five mulberry accessions being maintained in Kenya which include the accessions Embu, Thika, Thailand (*M. alba*), Kanva-2 and S41 (*M. indica*) using twelve phenotypic traits recorded from two localities, Nairobi and Eldoret. Leaf lamina width and petiole length, petiole width and growth height, internodes distance and number of

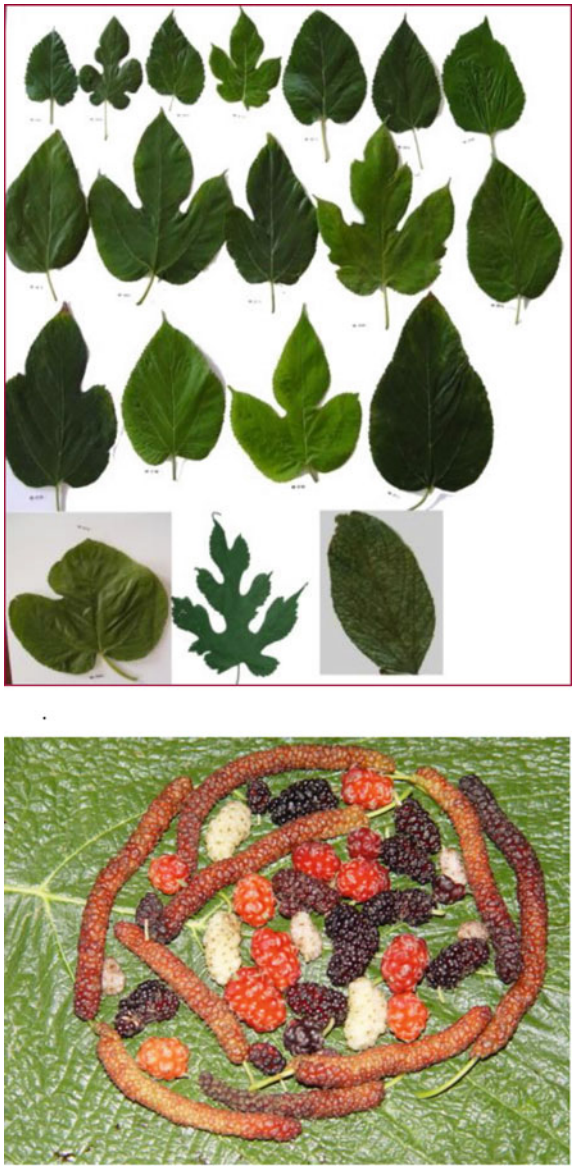

Fig. 7.4 Variation in the leaf and fruit morphology of mulberry

branches showed significant and using Duncan's Multiple Range Test (DMRT) the accessions were clustered into four groups.

Efforts were also made to evaluate the germplasm for variability on stress tolerance. Hossain et al. (1991) screened 10 mulberry genotypes under tissue culture condition to screen out the tolerant genotypes. Vijayan et al. (2003) screened 63 mulberry genotypes under in vitro conditions and selected 5 genotypes with higher

tolerance levels. Similarly, Tewary et al. (2000) screened mulberry genotypes for osmotic stress tolerance by imparting 1.0–10% polyethylene glycol (PEG) and observed considerable genetic diversity among the genotypes. These studies clearly showed that considerable genetic diversity is present in mulberry for stress tolerance. However, incorporation of the specific trait through conventional breeding has several bottle necks important among them is the difficulty in introgressing a trait from a donor parent to a recurrent parent through repeated back crossing and selection, due to the long juvenile period and dioecy of the plant (Vijayan 2010).

7.5.2 Genotype-Based Diversity Analysis

Since diversity analysis using morphological characters in plants are not very reliable due to the evolutionary dynamics, influence by the growing conditions and development stage, information from non-morphological characters such as nucleic acids and biochemical molecules is increasingly being used for genetic resource management and utilization. Among the non-morphological markers, molecular markers are ideal for genetic characterization of mulberry germplasm resources as they are highly polymorphic, multiallelic, codominant, non-episatic, neutral and insensitive to environment influence (Vijayan et al. 2004a). Although a number of DNA markers such as Random Amplified Polymorphic DNA (Xiang et al. 1995; Orhan et al. 2007), Amplified fragment length polymorphism (AFLP) (Huang et al. 2009), Inter simple sequence repeats (ISSR) (Vijayan and Chatterjee 2003; Vijayan et al. 2004a, b; Zhao et al. 2006, 2007a) have been developed and used for genetic diversity analysis of mulberry as shown in Table 7.11. However, considering the reproducibility, robustness, and information generating ability, simple sequence repeats (SSR), and single nucleotide polymorphism (SNP) markers are considered the most suitable molecular markers for genetic diversity analysis in mulberry. Simple sequence repeats (SSR) or microsatellite or short tandem repeat (STR) or simple sequence length polymorphism (SSLP) are tandem repeats of short (2–6 base pair) DNA fragments scattered throughout the genome that lies between conserved sequences (Litt and Luty 1989). The three mechanisms that create a new allele at SSR loci are (a) replication slippage (b) unequal crossing-over and (c) genetic recombination. Replication slippage is considered to be a major factor affecting the repeat number for short tandem repeat sequences, whereas unequal crossing-over is thought to result in a very large number of alleles for long tandem repeat arrays (Huang et al. 2002). However, the major disadvantage of SSR was the need genomic information to develop primers, which was expensive and time consuming, but with the introduction of Next generation sequencing technique, the cost has come down heavily and now it is possible to sequence any plant genome at a reasonable cost. Accordingly, a number of SSR markers have been identified in mulberry (Aggarwal et al. 2004; Zhao et al. 2005; Thumilan et al. 2013). Regarding SNPs, they are the most abundantly present DNA marker in any genome (Collins et al. 1997) and are single base variations widely distributed throughout the genomes with the frequency of >1% in a population

Table 7.11 Molecular markers used for genetic characterization in *Morus* spp. (adapted from Vijayan et al. 2018)

Sl. No.	Marker type	Purpose	Country	References
1	ISSR	Cultivar analysis for crop improvement	India	Vijayan and Chatterjee (2003)
2	ISSR, RAPD	Genetic diversity in mulberry (genus *Morus*)	India	Awasthi et al. (2004)
3	ISSR, RAPD	Phylogenic relationship among five species	India	Vijayan et al. (2004a)
4	ISSR, RAPD	Genetic diversity among wild populations of mulberry	India	Vijayan et al. (2004b)
5	ISSR, RAPD	Genetic relationship of Indian and Japan accessions	India	Vijayan (2004)
6	ISSR, RAPD	Genetic diversity among indigenous to India	India	Vijayan et al. (2005)
7	ISSR	Genetic relatedness among cultivated and wild mulberry	China	Zhao et al. (2006)
8	ISSR	Molecular characterization and identification of markers	India	Vijayan et al. (2006a)
9	ISSR, RAPD	Genetic relationships between wild and cultivated mulberry (*Morus*) species	India	Vijayan et al. (2006b)
10	RAPD	Genetic variability and phylogenetic relationship among 15 white mulberry genotypes	Turkey	Orhan et al. (2007)
11	ISSR	Genetic diversity among 66 local varieties belonging to 8 populations	China	Zhao et al. (2007a)
12	ISSR and SSR	Genetic diversity among 27 mulberry accessions including 19 cultivated and 8 wild accessions	China	Zhao et al. (2007b)
13	ISSR	Phylogenetic relationship among 18 germplasm collection and association with biochemical parameters	India	Kar et al. (2008)

(continued)

Table 7.11 (continued)

Sl. No.	Marker type	Purpose	Country	References
14	AFLP	Genetic variability among 43 accessions belonging to *M. alba*, *M. nigra* and *M. rubra*	Turkey	Kafkas et al. (2008)
15	ISSR	Genetic diversity among ecotypes	China	Zhao et al. (2008)
16	RAPD	Molecular characterization of inter and intra-specific hybrids	India	Tikader and Dandin (2008)
17	AFLP	DNA fingerprinting of ten cultivars	China	Huang et al. (2009)
18	ISSR and RAPD	Association with sprouting and sex expression traits	India	Vijayan et al. (2009b)
19	SRAP	Genetic diversity among 23 mulberry germplasm accessions from China	S Korea	Zhao et al. (2009)
20	RAPD	Phylogenetic relationship among 47 genotypes	Turkey	Ozrenk et al. (2010)
21	ISSR	Genetic diversity among 73 local mulberry varieties for development of core collection	China	Lin et al. (2011)
22	RAPD	Genetic variability among control and ethyl methane sulphonate (EMS) treated clones of mulberry genotype RFS135	India	Anil Kumar et al. (2012)
23	RAPD and ISSR	Genetic diversity and phylogenetic relatedness among 20 mulberry varieties	India	Chikkaswamy and Prasad (2012)
24	RAPD and ISSR	Genetic diversity among 20 mulberry varieties	India	Chikkaswamy et al. (2012)
25	RAPD and ISSR	Genetic diversity among 21 mulberry genotypes collected from 4 geographic regions of Turkey	Turkey	Ipek et al. (2012)

(continued)

Table 7.11 (continued)

Sl. No.	Marker type	Purpose	Country	References
26	RAPD	Genetic diversity among 36 genotypes collected from South India	India	Naik et al. (2013)
27	RAPD, ISSR and SSR	Standardization of novel and efficient DNA extraction protocol	India	Anuradha et al. (2013)
28	RAPD and ISSR	Genetic stability of cryo-preserved dormant buds of different Morus species belonging to indigenous and exotic collection	India	Choudhary et al. (2013)
29	SSR	Genetic diversity among 36 mulberry genotypes ('breeders' collections)	India	Krishnan et al. (2013)
30	RAPD	Genetic diversity among nine mulberry genotypes with contrasting traits for water use efficiency (WUE)	India	Mishra et al. (2013)
31	SSR	Genetic diversity among ten accessions belonging to *M. alba* and *M. indica*	Kenya	Wangari et al. (2013)
32	SSR	Phylogenetic relatedness among 17 mulberry genotypes	India	Wani et al. (2013)
33	SSR	Assessed the hybrid nature of two high yielding mulberry varieties	India	Arora et al. (2014)
34	RAPD, ISSR and SSR	Genetic diversity among 850 germplasm accessions collected from 23 countries for development of core collection of diverse accessions	India	Krishnan et al. (2014a)
35	SSR	Genetic diversity in wild mulberry species of India	India	Naik et al. (2015)
36	ISSR, RAPD	Genetic diversity and relationship of mulberry		Banerjee et al. (2016)

(continued)

Table 7.11 (continued)

Sl. No.	Marker type	Purpose	Country	References
37	RAPD and ISSR	Genetic fidelity of in vitro regenerated mulberry plants (cv. S1)	India	Saha et al. (2016) Rohela et al. (2018, 2020)
38	SSR	Characterization of Genic SSR Markers from Transcriptome and their Transferability to Related Species of Moraceae	India	Thumilan et al. (2016)
39	SSR	Association mapping of Charcol root rot diseases	India	Pinto et al. (2018a)
40	RAD-Seq	Identification of dominant genetic markers relevant to male sex determination in mulberry	Japan	Atsumi et al. (2019)
41	SSR	The molecular characterization of an extended mulberry germplasm by SSR markers	Spain	Garcia-Gómez et al. (2019)
42	SSR	Molecular characterization of mulberry genotypes and species in Turkey	Turkey	Orhan et al. (2020)
43	SNP	Phylogenetic analysis of eight species of Morus	Japan	Muhonja et al. (2020)
44	SSR	Genetic diversity, identification and utilization of novel genetic resources for resistance to *Meloidogyne incognita* in mulberry	India	Arunakumar et al. (2021)

(Halushka et al. 1999). The frequency of SNPs is roughly estimated to be one in every thousand nucleotides in the human genome, and one in 60–120 bp in maize (Ching et al. 2002). However, to date, no attempt was made to discover SNPs in mulberry. Nonetheless, considering the tremendous progress made on low-cost and high-throughput SNP genotyping in other crops, and the current pace of genomic research in mulberry, it is certain that within a short time SNPs become the commonly used molecular markers in mulberry. A large number of ESTs from mulberry genome have been deposited in the data bank (Lal et al. 2009; Zhao 2008). Attempts should, therefore, been made to identify potential SNPs from these ESTs, which can also

be used for identifying casual polymorphism. Likewise, SNPs can also be developed through locus specific amplification (LSA) and comparative re-sequencing from multiple individuals (Rieder et al. 1998) by utilizing the information available from the genomic sequences deposited from markers like ISSR and RAPD that are associated with valuable phenotypic traits.

7.5.3 Relationship with Other Cultivated Species and Wild Relatives

Usage of crop wild relatives (CWRs) in cultivation and breeding is the best way to harness natural trait variation in genetic improvement programs. Wild relatives often have unique alleles for specific traits like resistance to pest and diseases and tolerance to abiotic stresses. Thus, it is desirable to understand the relationship between wild and domestic species in order to plan the crop improvement programs effectively. The phylogenetic relationship among different genera of the family Moraceae was generated with information from nuclear and chloroplast DNA sequence variations of thirteen species of *Morus* distributed in Asia, Africa, Europe, and North, Central, and South America. The study revealed that the genus *Morus*, as currently circumscribed, is non-monophyletic as the species *M. mesozygia* and *M. insignis* are placed outside the other domestic species. Thus, further detailed investigation is required to clarify natural generic relationships of the family Moraceae (Nepal and Ferguson 2012). Vijayan et al. (2004a) used inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers to find out the relationship among five species viz., *M. latifolia*, *M. bombycis M. alba* and *M. laevigata* and found that *M. laevigata* is different from other species. Population analysis further stressed the wild nature of *M. laevigata* as it showed considerably low gene flow (Nm) with other species. Likewise, Weiguo et al. (2007) using inter-simple sequence repeats (ISSR) and simple sequence repeat (SSR) markers investigated the genetic diversity among 27 mulberry accessions including 19 cultivated accessions (six *M. multicaulis*, three *M. alba*, two *M. atropurpurea*, two *M. bombycis*, one *M. australis*, two *M. rotundiloba*, one *M. alba* var. *pendula*, one *M. alba* var. *macrophylla*, and one *M. alba* var. *venose*) and 8 wild accessions (two *M. cathayana*, two *M. laevigata*, two *M. wittiorum*, one *M. nigra* and one *M. mongolica*). It has found that long history of cultivation has caused loss of genetic diversity in domestic species (Jiao et al. 2020).

7.5.4 Relationship with Geographical Distribution

Although not much information is available on the relationship between geographic distribution of mulberry and the genetic diversity among the accessions, Vijayan and Chatterjee (2003) analysed the relationship between geographic distribution and

genetic diversity among 11 cultivars of Indian origin and found that the cultivars grouped as per their geographic origin. Likewise, Chatterjee et al. (2004) found a strong relationship between geographic distribution and genetic diversity of *M. laevigata.* Likewise, Vijayan et al. (2005) assessed the genetic diversity among 34 Indian mulberry accessions and found that the accessions resolved into groups based on their geographic relationships. Efforts to find out the relationship among cultivars originated in India and Japan also showed clear and distinct grouping based on their geographic origin (Vijayan 2004). Jiao et al. (2020) used population genomic analysis by resequencing 132 mulberry accessions split domesticated mulberry into three geographical groups, namely, Taihu Basin of southeastern China (Hu mulberry), northern and southwestern China, and Japan, among which Hu mulberry had the lowest nucleotide diversity and demonstrated obvious signatures of selection indicating environmental adaptation. Thus, mulberry varieties and cultivars from different geographic regions show high genetic diversity.

7.5.5 Extent of Genetic Diversity

The extent of genetic diversity within a species was investigated in *M. alba* and *M. serrata* with ISSR and RAPD markers. It is found that the genetic similarity among 11 mulberry genotypes of *M. alba* viz., Limoncina, Schinichinose, Kattaneo, Obawasa, Rangoon, China white, China black, Canton china, Almora local, Punjab local and Sujanpur-2 estimated based on Nei and Li (1979) varied from 0.644 between Rangoon and Punjab local to 0.943, between Sujanpur-2 and Almora local, with an average genetic similarity of 0.793 for data generated by ISSR markers. The same was in the range of 0.738 for China white and Kattaneo to 0.909 for Sujanpur-2 and Punjab local with an average of 0.834 in RAPD analysis. When the ISSR and RAPD data were pooled to analyse with more markers the genetic similarity among the genotypes varied from 0.733 between China white and Kattaneo to 0.888 between Sujanpur-2 and Punjab local with a mean coefficient of 0.819. The genetic similarity coefficients revealed a substantial amount of genetic similarity among the genotypes, though the genotypes were collected from different countries of much varied climatic conditions such as tropical, temperate and subtropical. Genotypes from similar geographic regions showed closer genetic similarity than those from geographically distant region. The correlation coefficient among the matrices as tested by Mantel (1967) Z-statistics, revealed high correlations ($r = 0.4$; $p = 0.000$ between ISSR and RAPD; $r = 0.976$, $p = 0.000$ between ISSR and Pooled matrices, $r = 0.982$; $p = 0.000$ between RAPD and Pooled data matrices) (Srivastava et al. 2004). The genetic diversity evaluation of 16 populations of *Morus serrata* Roxb., revealed the presence of significant genetic diversity among the populations on morpho-anatomical traits as well as in DNA markers. The average genetic distance, estimated from the ISSR markers was 0.165 (Vijayan et al. 2006a). Thus, there is a great amount of inter and intraspecies genetic diversity in mulberry which can be used for crop development.

7.6 Association Mapping Studies

7.6.1 Extent of Linkage Disequilibrium

Association mapping is the method of identifying molecular markers that are tightly linked to complex phenotypic traits based on significant allele-frequency differences between individuals with the phenotype of interest ("cases") and a set of unrelated control individuals (Farnir et al. 2000). The major advantage of association mapping over traditional biparental linkage mapping is the exploitation of historical and evolutionary recombination events. The extent of linkage disequilibrium is one of the important factors that decide the success of association. Linkage disequilibrium (LD) or the gametic disequilibrium is the non random association of alleles at different loci leading to inherit jointly during meiosis than the alleles of loci farther apart (Mackay and Powell 2007). LD reflects the proximity of the loci and the low probability of recombination to take place between the loci to break the association between them. LD was described by Jennings (1917) and later estimated by Lewontin (1964) based on the differences between the observed and expected frequencies of alleles. LD is influenced by several factors, including genetic drift, admixture of populations, mutations and recombinations in the genome, natural selection, population size, and other genetic events. Many methods are now available to estimate the LD in a population, but the most frequently used LD measures are D or D′ [($D = P_{AB} - P_A P_B = P_{AB}P_{ab} - P_{Ab}P_{aB}$), where P_A and P_B are allele frequencies], and r^2 (Hartl and Clarke 1989). The D′ is the standardized disequilibrium coefficient which mainly measures recombinational history and is, therefore, useful to assess the probability of historical recombination in a given population. This statistic may be affected by sample size (with smaller size samples demonstrating larger D'_m values) and allele frequencies (Mohlke et al. 2001) and therefore may not be appropriate for comparison of LD between studies that use different sample sizes and markers with differing numbers of alleles and differing allele frequencies. Another commonly used method is the estimation of r^2, which is essentially the correlation between the alleles at two loci; it summarizes both recombinational and mutational history (Hill 1974). The r^2 values are the square of the Pearson correlation coefficients, and are related to D by $r^2 = D^2/[p_A(1 - p_A)p_B(1 - p_B)]$. The r^2 value of equal to 0.1 (10%) or above is considered as significant threshold for the rough estimate of LD to reveal association between pairs of loci. There are two common ways to summarize and visualize the LD across a region. LD decay plots are used to visualize the rate at which LD declines with genetic or physical distance while disequilibrium matrix is used for visualizing the liner arrangement of LD between polytrophic sites within a gene or loci along the chromosome. There are many computer software that can be used for visualizing linkage disequilibrium (Table 7.12). Thus, in Linkage disequilibrium (LD) mapping, instead of relying on data from a specific experimental cross or pedigree, the natural genetic variability present in the species or in a particular population is exploited as it is the cumulative effect of a number of natural crosses among the members. LD mapping offers three distinct advantages. (1)

Table 7.12 Commonly available softwares for visualization of linkage disequilibrium

Software	Available from
GOLD	http://www.sph.umich.edu/csg/abecasis/GOLD
GRR	http://www.sph.umich.edu/csg/abecasis/GRR/
GOLDsurfer	http://www.umbio.com
HAPLOPAINTER	http://haplopainter.sourceforge.net/html/ManualIndex.htm
HAPLOT	http://info.med.yale.edu/genetics/kkidd/programs.html
HaploView	http://broad.mit.edu/mpg/haploview/index.php
HaploVisual	http://www.cs.helsinki.fi/u/prastas/haplovisual/
JLIN	http://www.genepi.com.au/projects/jlin
LDheatmap	http://stat-db.stat.sfu.ca:8080/statgen/research/LDheatmap
LDMAP	http://cedar.genetics.soton.ac.uk/pub/PROGRAMS/LDMAP
Marker	http://www.gmap.net/marker
PowerMarker	http://statgen.ncsu.edu/powermarker/index.html
SNPanalyzer	http://www.istech.info/istech/board/login_form.jsp
TASSEL	http://www.maizegenetics.net

Provides better mapping resolution by exploiting higher number of meiotic recombination. (2) Since LD mapping harness the genetic diversity of natural populations or germplasm to potentially resolve complex trait variation to single gene or nucleotides, it reduces the research time taken for developing expensive and tedious biparental populations that are essential for classical linkage mapping. (3) Since LD mapping uses information from the whole population, a broader genetic variation in a more representative genetic back ground is available. Thus, one does not depend on the marker-trait relationship that occurs only between two specific parents. LD mapping can attain high resolution due to the utilization of recombinations that have taken place since the founder population (Yu and Buckler 2006). Additionally, past data on phenotypic characters can be used for linking markers to the trait, so that new trials need not be carried out again. LD maps are also useful for the identification of genomic regions that have undergone differential selection (between populations) and related phenomena (Abdurakhmonov et al. 2008). In addition to being useful for QTL mapping, association mapping sometimes helps identify mutations that cause specific phenotypes (Palaisa et al. 2004). The extent of LD may vary greatly among species and within genomic regions and it may extend over long physical distances,

for example the extensive LD seen in species like *Arabidopsis thaliana* or in inbred lines of barley, where LD can extend for tens or even hundreds of kilobase pairs, allow for genome-wide association mapping with a relatively low number of evenly spaced SNPs markers, but plants like mulberry which are predominantly or obligately outcrossing and have long juvenile periods, LD may extend only a few hundred base pairs at the most and for adequate genome-wide coverage several million SNPs may be required.

7.6.2 Target Gene Based LD Studies

Target-gene based association mapping relates polymorphisms in selected targeted genes that play a key role in controlling the phenotype of specific traits (Zhu et al. 2008). In general, target gene based association mapping is easy for simple biochemical pathways and traits controlled by a few genes but for complex traits, the whole genome could potentially serve as a target gene (Yu and Buckler 2006). One of the easy, reliable, and rational methods of target gene selection is the utilization of expressed sequence tag (EST) databases of the crop. ESTs are short (~200–700 nucleotides) subsequences of transcribed and spliced DNA. Since EST based genome scan concentrates on coding regions, it can discover target genes for several complex traits including adaptation to divergent climatic conditions. Once the target gene is identified, a chromosome segment of at most a few megabases that has strong association with the phenotype is identified. Genetic markers with very high frequency like SNPs, which are potentially in LD with causative polymorphism, are ideal for target gene mapping. The rate of LD decay for a specific target gene locus dictates the number of markers per unit length needed to identify significant associations. Since SNPs present in the coding regions often cause phenotypic variation; they should be given top priority while selecting markers for target gene mapping. In mulberry not much work has been done on target based LD mapping.

7.6.3 Genome Wide LD Studies

Genome wide association mapping or gene scan surveys genetic variation in the whole genome to locate genes or narrow regions that have significant statistical connections with various complex traits. Since to conduct a genome wide association analysis, an enormous number of densely distributed markers is required, whole genome scan is usually carried out using the most frequent genetic variants available in the genome that is single nucleotide polymorphisms (SNPs). Generally, thousands of SNP markers are required for whole genome scan for crops with low LD and high haplotype diversity. Root rot is a serious disease in mulberry and causes severe economic losses (Gnanesh et al. 2021), recently Pinto et al. (2018a) used a germplasm panel of 214 germplasm accessions to identify markers associated with charcoal root

rot resistance and identified five AFLP markers associated with root rot resistance. These markers accounted for 9.6–12.7% of the total phenotypic variation in the trait (R^2) and had an allele frequency of 0.132–0.401. Similarly, Zhang et al. (2016) used a germplasm panel of 93 mulberry accessions of diverse origin was to identify markers for a few important fruit traits. A total of 24 markers associated with fruit traits were identified. Thus, very scanty work only has been done in mulberry on LD mapping.

7.6.4 Future Potential for the Application of Association Studies for Germplasm Enhancement

LD mapping is very useful for identification trait-marker associations in species where biparental mapping has limitations, especially crops like mulberry where inbreds are hardly available. Mulberry being a perennial species, association mapping is very appealing as large populations of trees, comprising of wild species, weedy species, land races and modern cultivars, are important reservoirs of natural genetic variations, originated from a number of historical genetic events as a response to different climatic conditions. A wide range of genetic variation, exist in the germplasm collections and commercial plantations (Tikader and Vijayan 2017). The exploitation of these genetic variability in the ex situ conserved genetic resources is vital to overcome future problems associated with narrowness of the genetic base of modern cultivars as strong genetic diversity means diverse morphological traits and a higher potential to develop varieties for varied cultural and agronomic conditions (Abdurakhmonov and Abdukarimov 2008). Since LD analysis has the potential to identify a single polymorphic locus within a gene that is responsible for a difference in phenotype and to predict the best haplotype across one or multiple genes for optimum expression of the target trait, it can be used to determine the best donor parents for crop improvement programs. The current efforts to sequence the genome of diploid mulberry species in India and to identify SNPs would help perform more association mapping as a biallelic codominant type of markers like single nucleotide polymorphisms (SNPs) is perfectly suitable for the quantification methodology of LD. LD quantification using dominant markers such as RAPD, AFLP, ISSR is poorly explored and usually subject to wrong perception and interpretation. Another important factor that determines the success of LD mapping is the choice of germplasm or population (Yu et al. 2006) as the false positives generated by population structure may make a marker allele that occurs at high frequency in a preferentially sampled subpopulation associate with trait of interest even though it is not linked to a real QTL (Pritchard et al. 2000). To overcome these problems, a number of methods such as structured association, mixed model approach, genomic control and principle component approach have been developed (Devlin and Roeder 1999; Pritchard et al. 2000; Yu et al. 2006). Thus, the true potential of LD mapping is yet to be harnessed in mulberry.

7.7 Brief Account of Molecular Mapping of Resistance/Tolerance Genes and QTLs

7.7.1 Brief History of Mapping Efforts

Mulberry breeding thus far focused on the improvement of leaf yield and its component characters through selection on phenotypes and most of the agronomical traits are quantitative and are controlled by polygenes and "multifactorial" and their expression is highly influenced by environment and growth stages. Thus, it would be easier to improve these traits through indirect selection based on trait-linked molecular markers, otherwise called marker assisted selection breeding. Molecular markers are the DNA sequences that have been used as genetic markers to identify the individual, species and marker-trait association. Hence, these markers similar to genes can be mapped on chromosome by using the principle of genetics involving law of independent assortment and linkage analysis. The recombination frequency between two genes is directly related to the distance between two markers/genes and determines in term of centi Morgan (1 cM = 1% recombinants). In annual and biannual plants where the generation period is less, genetic linkage maps are constructed from segregating populations such as F2, back crosses (BC), doubled haploids (DH), recombinant inbred lines (RILs) and near isogenic lines (NILs) but in trees like mulberry homozygous lines are difficult to develop due to the high genetic load and the long generation period. Hence, in such cases, the linkage map is developed from F1 progenies, either full-sibs or half sibs, using "pseudo-testcross strategy" (Grattapaglia and Sederoff 1994). The assumption behind this method is that in a cross between two parents the markers in heterozygous condition in one parent and null in the other segregate in 1:1 ratio in the progeny (Grattapaglia and Sederoff 1994). However, this technique has several inherent limitations the most important one is the individual-specific nature of the map. Therefore, for every elite breeding line, separate maps have to be developed. Integration of map is possible if multiallelic codominant markers like microsatellite markers are used. Two other methods that have recently been used for constructing linkage maps in tree plants are the "F2 inbred model" and the "three-generation outbred model". In the F2 inbred model, pedigree information for three generations is harnessed by considering the grandparents as inbred lines (represented by A1/A1 and A2/A2). In the F2 generation, three genotypes occur at any loci: A1/A1, A1/A2, and A2/A2, segregating 1:2:1. Since the F2 progeny of this strategy theoretically corresponds to the classical F2 progeny, MAPMAKER can assemble a combined parental map from the F2 progeny data using the intercross mating type. The three-generation outbred model is an extension of the pseudo-testcross strategy wherein co-dominant markers such as SSR markers are used for analysis. In this case, if one parent is heterozygous and the other is homozygous the segregation will be 1:1, on the other hand, if both parents are heterozygous then the segregation will be either 1:2:1 and the segregation will be 1:1:1:1 when both parents have different genotypes. Later, Margarido et al. (2007) developed One Map for facilitating linkage map construction in outcrossing plants like mulberry wherein full-sib families derived from two

outbred parents can be used. The analyses are performed using a novel methodology based on the maximum likelihood approach for simultaneous estimation of linkage and linkage phases using analysis of a mixed set of different marker types containing various segregation patterns, such as 1:1:1:1, 1:2:1, 3:1 and 1:1.

7.7.2 Evolution of Marker Types

Since the ideal marker for genetic analysis of mulberry should be polymorphic, multiallelic, codominant, non-episatic, neutral and insensitive to environment and morphological markers do not meet these criteria, efforts have been made to develop or use biochemical and molecular markers in mulberry. The first attempt was with Isozymes, which are multiple forms of enzymes that share a common substrate but differ in electric mobility. However, due to the low polymorphism baring a few sporadic attempts of genetic diversity analysis in mulberry (Hirano and Naganuma 1979; Hirano 1982; Venkateswarlu et al. 1994; Rao et al. 2011) not much work has been done with isozymes in mulberry. Side by side efforts has been made to utilize molecular markers. Restriction fragment length polymorphism (RFLP) markers are the first generation of DNA markers and it is based on digestion of the DNA with restriction enzymes and identifying the DNA fragments through digestion with restriction enzymes, electrophoresis on a gel and Southern-Boltting with probes. Since, RFLP needs large quantity of high quality DNA for restriction enzyme digestion and it is difficult to extract such high purity DNA from mulberry due to the high phenolic contents in the leaf, RFLP become unpopular in mulberry (Vijayan 2007). The next and most widely used DNA marker was Random amplified polymorphic DNA, which used arbitrary short oligomers (usually 10-mer) to anneal to random homologous target sites within the genome to generate polymorphic markers arising from base pair substitutions modifying the primer binding sites. Insertions in the genomic sequence that separate the primer binding sites to a distance that prohibit amplification or cause length changes of the amplified product results in RAPD profile variation among individuals (Moeller and Schaal 1999). RAPD was mainly used for the characterization of genetic resources (Xiang et al. 1995; Bhattacharya and Ranade 2001; Mishra et al. 2013; Banerjee et al. 2016). Side by side Inter simple sequence repeat (ISSR) and Amplified Fragment Length Polymorphism (AFLP) markers were used for germplasm characterization and biodiversity analysis of mulberry (Sharma et al. 2000; Wang and Yu 2001; Vijayan et al. 2006a, b; Banerjee et al. 2016). Since these markers are dominant, anonymous and suffering from fidelity, efforts were made to develop more robust, informative and reproducible marker systems. Simple sequence repeats (SSR) or microsatellite or short tandem repeat (STR) or simple sequence length polymorphism (SSLP) is one such marker system that has got wider acceptance. Genic and genomic SSR markers were developed and used for several studies as shown in Table 7.11. With the advent of next generation sequencing, it has been found that vast majority of polymorphisms that exist in the DNA sequence are single base pair differences, thus the focus has

shifted to develop makers based on these single nucleotide polymorphism (SNPs). SNPs are found in both transcribed and non-transcribed regions and the less likelihood of occurrence of cross over between two adjacent SNPs in comparison with other markers, and the easiness with which automation can be applied for SNPs, this marker system has recently gained wider acceptance for linkage mapping and biodiversity assessments. In mulberry, many efforts are being made for genome-wide SNP discovery and Muhonja et al. (2020) through double-digest restriction site-associated DNA sequencing (ddRAD-seq), identified 2229 homozygous SNPs of 54 mulberry varieties in the eight species and used for phylogenetic analysis.

7.7.3 Mapping Populations Used

In recent years much emphasis has been made to develop biparental based linkage maps in mulberry as result a large number of mapping populations have been developed from parents with contrasting traits. Venkateswarlu et al. (2006) developed a mapping population of 369 F1 hybrids from a cross between V1x S36 and used for pseudotest cross linkage map analysis. Similarly, Thumilan et al. (2016) developed a mapping population of 150 F1 hybrids from a cross between Dudia White × UP 105 and used for linkage map construction using SSR markers. Mishra (2014) used 200 F1 hybrids developed from Himachal Local × MS-3 for identification of QTLs for Water use efficiency in mulberry and 560 F1 from Dudia White x UP and 35 F1 from Punjab Local × UP for QTL mapping of root traits.

7.7.4 Mapping Software Used

The most important application of molecular markers is to locate the genomic regions on chromosome that affect phenotypic expression of a desirable trait. Identification of such genomic regions is possible with or without a molecular map. The QTL is identified through estimation of the level of association between the molecular marker and the trait in a segregating population such as F_2, backcross (BC), recombinant inbred lines (RILs), and double haploid lines. Several software packages are available to construct molecular maps from biparental mapping populations. The following are some of the important ones being used widely (Table 7.13).

7.7.5 Maps of Different Generations

In mulberry, not many genetic linkage maps have been constructed as shown in Table 7.14. Nonetheless, the beginning of the genetic map construction in mulberry was started by Venkateswarlu et al. (2006) with 50 F1 full-sib progeny using randomly

Table 7.13 Some of the useful softwares for linkage mapping and QTL detection

Software	Availability	References
MAPMAKER	http://www.broad.mit.edu/ftp/distribution/software/mapmaker3/	Lander et al. (1987)
JOINMAP	http://www.kyazma.nl/index.php/mc.JoinMap/	Stam (1993)
MENDEL	http://gnome.agrenv.mcgill.ca/info/gmendel.htm	Echt et al. (1992)
OneMap	http://www.ciagri.usp.br/aafgarci/OneMap	Margarido et al. (2007)
THREaD Mapper Studio	http://cbr.jic.ac.uk/threadmapper	Cheema et al. (2010)
MAP Manager QTX	http://mapmgr.roswellpark.org/mmQTX.html	Matsui et al. (2010)
CARTHAGENE	http://www.inra.fr/mia/T/CarthaGene/	de Givry et al. (2005)
MAPMAKER-QTL	http://www-genome.wi.mit.edu/genome_software	Lander and Botstein (1989)
MCQTL	http://carlit.toulouse.inra.fr/MCQTL/	Jourjon et al. (2005)
MapQTL	http://www.mapqtl.nl	Van Ooijen (2004)
PLABQTL	https://www.uni-hohenheim.de/plantbreeding/software/	Utz and Melchinger (1996)
QTLExpress	http://qtl.cap.ed.ac.uk/	Seaton et al. (2002)
QTL Cartographer	http://statgen.ncsu.edu/qtlcart/cartographer.html	Basten et al. (1998)
Qgene	http://www.qgene.org/	Nelson (1997)

Table 7.14 Molecular linkage map developed in mulberry (*Source* Sarkar et al. 2017)

Sl. No.	Molecular map	Pedigree of mapping population	Markers	Hereditary nature of marker	Agronomic trait targeted	References
1	Genetic linkage	S36 × V1	RAPD, ISSR, and SSR	Dominant, co-dominant	No	Venkateswarlu et al. (2006)
2	QTL map	V1 × Mysore Local	RAPD and ISSR	Dominant	Yield traits	Naik et al. (2014)
3	QTL map	Himachal Local × MS3	RAPD and ISSR	Dominant	Water use efficiency	Mishra (2014)
4	QTL map	Dudia White × UP	RAPD and ISSR	Dominant	Root traits	Mishra (2014)
5	Genetic linkage	Dudia White × UP105	SSR	Co-dominant	No	Thumilan et al. (2016)

amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), and simple sequence repeat (SSR) markers using pseudo-testcross mapping method. Using 517 markers generated with RAPD, ISSR, and SSR primers two separate female and male maps were constructed containing 12 female linkage groups and 14 male linkage groups. The threshold LOD score was 6.0 and the maximum map distance was 20 cM. The female map covered 1,196.6 cM distance, with an average distance of 15.75 cM and maximum map distance of 37.9 cM between two loci. The map of the male parent covered a 1,351.7-cM distance, with an average distance of 18.78 cM and a maximum map distance between two loci is of 34.7 cM. The markers distributed randomly in all linkage groups without any clustering. Subsequently, Thumilan et al. (2016) developed another genetic map with 453 SSR markers including 264 EST-SSRs. Genetic linkage map was construction with 262 (57.83%) markers showing test cross segregation. The markers covered a total map distance of 4263.5 cM on 14 linkage groups. The distribution of the markers was random and unequal between linkage groups and clustered in some regions (LG2 and LG4). The distance between two linked markers varied from 5.0 cM between MUL3SSR140 and MUL3SSR158 to 49.8 cM between MESTSSRCP243 and MESTSSRCP267 on LG4. The average map distance observed between two linked markers was 31.8 cM. The largest linkage group LG4 had map coverage of 1923.6 cM with 51 markers followed by LG2 with 33 markers spanning 1325.5 cM. In another effort, Mishra (2014) constructed a linkage map for water use efficiency in mulberry using 200 F1 hybrids of Himachal local × MS-13. A total of 20 linkage groups on Himachal Local (♀parent) map and 16 on MS-3 (♂parent) map were established. Approximately 63% of the markers could be placed on the framework linkage map of Himachal Local defining a total of 60 loci or loci clusters and covering a total map distance of 2374.7 cM. Similarly, on MS-3 map, about 61% of the markers could be placed on a framework of 60 loci or loci clusters covering 1327.2 cM. The average size of linkage groups in Himachal Local was 118.74 ± 169 cM and the length ranged from 2.1 to 700.5 cM. However, in MS-3 the average size of the linkage groups was 82.95 ± 60 cM and ranged from a minimum of 1.6 to a maximum of 95.9 cM. The average distance between two framework markers was 39.57 ± 15 cM on Himachal Local and 22.12 ± 16 cM on MS-3 map.

7.7.6 Details on Trait Wise QTLs

Using the above maps, Mishra (2014), identified several QTLs for WUE using the software WinQTL cartographer version 2.5. QTL for length of the longest shoot (LLS), inter-nodal distance (IND), and average shoot length (ASL) were identified in Linkage group 9 of the female parent. A QTL for shoot weight was detected on Linkage group 7 of female parent (R2 = 13.8%) and a QTL for longest root length was located on Linkage group 5 of the same parent flanked by I041500 and K011750 markers. A cumulative of 36 QTLs associated with root and WUE traits

were identified and available for validation (Madhuri 2015). Similarly linkage and QTL mapping have been reported for yield relevant traits in mulberry (Venkateswarlu et al. 2006; Naik et al. 2014). Other than these few work, not much work has been done in mulberry to identify QTLs.

7.8 Marker-Assisted Breeding for Resistance/Tolerance Traits

7.8.1 Germplasm Characterization and DUS

Several molecular markers have been used for characterization of germplasm as stated elsewhere. Biochemical markers such as isozymes viz. peroxidase (PRX), α and β esterase (EST), amylase (AMY), acid phosphatase (ACP), malate dehydrogenase (MDH), superoxide dismutase (SOD), polyphenol oxidase (PPO) and diaphorase (DIA), were used to elucidate the genetic diversity among 14 mulberry species (Rao et al. 2011). Similarly**,** DNA markers such as random amplified of polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP), Intersimple sequence repeats (ISSR), and Simple sequence repeats (SSR) were used for assessing the genetic diversity among mulberry genotypes (Table 7.11). Pinto et al. (2018b) used a panel of 216 accessions and 24 SSR primers for the Distinctness, uniformity, and stability (DUS) analysis of mulberry. A total of 91 rare alleles and two common alleles along an average dissimilarity value of 0.547 were observed. The resolving power of the SSR markers was in the range of 0.11 (MulSatG100717) to 3.67 (MulSatG92497), with an average of 2.13 and totaling to 51.18. The information generated in the above studies was used to develop DUS for mulberry along with phenotypic traits.

7.8.2 Marker-Assisted Gene Introgression

In mulberry baring a few limited attempts, not much work has been done using marker assisted selection breeding due to paucity of genomic information, high heterozygosity, outbreeding nature with a long generation cycle. Mishraet al. (2013) used molecular markers to introgress WUE and root traits with the help of QTLs identified through SSR markers. They assessed eight gas exchange parameters {viz. photosynthetic rate (A), conductance (gs), intercellular CO_2 concentration (Ci), transpiration rate (Tr), leaf vapor pressure deficit (vpd), leaf temperature (Tleaf), ratio of assimilation rate and stomatal conductance (A/gs) and ratio of intercellular carbon concentration and stomatal conductance (Ci/gs) in 63 introgressed hybrids and found five hybrids have better traits and confirmed presence of QTLs for such traits in them. Further, Arora et al. (2015) used RAPD and SSR markers to assess the hybrid vigour of the mulberry variety C2038.

7.8.3 Gene Pyramiding

The objective of many breeding programs is to improve several agronomic and economic traits such as yield, quality, and resistance to both biotic and abiotic stresses simultaneously. To achieve such improvement for multiple traits is very challenging and depends not only on the availability of appropriate genetic resources but also the knowledge on the genetic base of the traits and the genetic relationships among the traits. Past efforts using conventional breeding program proved that it is near impossible in mulberry to do such exercise due to the very nature of the plant. Hence, most of the breeding schemes end up in developing F1 progenies from selected parents and screening the progenies for the best genetic combinations and subsequent clonal propagations. However, the recent developments in molecular marker technologies enabled the breeders to change their strategies and concentrate more targeted breeding through marker assisted selection, as it helps to facilitate easy screening of a large number of progenies for the desired trait without subjecting them into the biotic and abiotic stresses. Marker-assisted gene pyramiding is one of the easy techniques to introgress single or multiple genes into a variety in the shortest period. However, in mulberry, no such attempts have been made so far due to the lack of adequate information on genes and markers-trait associations and also due to the breeding behavior of the plant.

7.8.4 Limitations and Prospects of MAS

Mulberry being a perennial tree with long generation times, high heterozygosity, out crossing breeding behavior, poor juvenile-mature trait correlations, and polygenic nature of most of the important traits posed many challenges to conventional breeders. Thus, marker-assisted selection (MAS) is considered a tool to accelerate breeding through early selection, especially for abiotic stresses. MAS depends on identifying DNA markers that are tightly linked to the trait of interest. As stated above, although a few genetic linkage maps have been developed using biparental mating with pseudo-test cross strategy, none of these genetic maps and subsequent efforts were able to identify validated QTLs to be used in breeding program in mulberry. The major limitations of the above efforts include the sparse distribution of markers, low resolution of marker-trait associations, the small proportion of phenotypic variation explained by QTL and the low success rate in validating QTL in different genetic backgrounds and environments. Further, to develop high resolution maps to identify markers with a tight association, more abundantly available markers like Single nucleotide polymorphisms (SNPs) have to be developed. Such effort is currently in progress at different research organizations across the globe.

7.9 Map-Based Cloning of Resistance/Tolerance Genes

7.9.1 Traits and Genes

Functional genomics in mulberry is still in its infant stage as not much work has been carried out in this area, barring a few attempts to develop expressed sequence tags (ESTs) associated with stress tolerance (Checker et al. 2012). ESTs developed from clones and unigenes identified through transcriptome analysis serve as sources of information for gene and trait identification. In mulberry, EST data sets were developed from leaf (Gulyani and Khurana 2011) and root (Checker et al. 2012) of the Indian mulberry species *M. indica, M. laevigata* and *M. serrata.* Comparison among these data sets helped identify novel/trait specific genes (Saeed et al. 2016). Genes involved in 1-deoxynojirimycin (DNJ) biosynthesis (Wang et al. 2018), Helix–Loop–Helix-144 (bHLH144), remorins, Dehydration-15 (ERD15), Nitrite Reductase, chalcone synthase has been found involved in stress tolerance (Checker and Khurana 2013; Wang et al. 2014b, 2017b; Sajeevan and Nataraja 2016; Saeed and Khurana 2016). Genome-wide scanning identified the transcription factors NAC and WRKY play a role in biotic and abiotic stress responses (Baranwal and Khurana 2016; Baranwal et al. 2016). Seventeen ABA-dependent signaling pathway-related genes including five *MnPYL*, six *MnPP2C*, and six *MnSnRK2* genes were identified from the genome sequences of *M. notabilis* and qPCR analysis revealed higher expression of *MaPYL4*, *MaPP2C5*, and *MaSnRK2.6* during fruit development and *MaPYL1/3/5*, *MaPP2C2/4/5/6*, and *MaSnRK2.3/2.6* during the post-maturation stage in *M. atropurpurea* cv. Jialing No. 40 (Zhu et al. 2017). Further, a microRNA, the miR166f, enhanced tolerance of a transgenic mulberry to drought stress (Li et al. 2018a). Further, Ukaji et al. (1999) observed accumulation of *WAP27A* and *WAP27B* proteins in the cortical parenchyma cells of mulberry tree (*M. bombycis* Koidz.) during winter conferring tolerance to cold. Sun et al. (2020) demonstrated the role of a chloroplast drought-induced stress protein (*MaCDSP32*) in conferring drought tolerance to mulberry. Likewise, Chen et al. (2018) observed strong interaction between *mAKR2A* and *mSOD1* suggesting a role of *mAKR2A* in the development of transmembrane proteins and cytoplasmic proteins under cold conditions. Similarly, Liu et al. (2015) observed the transcription factor MnDREB4A conferring enhanced tolerance to multiple abiotic stresses. In order to elucidate genomics of fruit ripening in mulberry, quantitative real-time polymerase chain reaction was performed with 17 genes participating in ABA signal transduction including five *MnPYL*, six *MnPP2C*, and six *MnSnRK2* genes, isolated from the *M. notabilis* genome database in *M. atropurpurea* cv. *Jialing* No. 40 and found that the transcripts of *MaPYL4*, *MaPP2C5*, and *MaSnRK2.6* were expressed at a relatively higher level during the entire development process. Similarly, the transcript levels of *MaPYL1/3/5*, *MaPP2C2/4/5/6*, and *MaSnRK2.3/2.6* were lower during the early-maturation stage and higher during the post-maturation stage, suggesting that they may play a vital role in regulating mulberry fruit ripening (Zhu et al. 2017). Efforts are, thus, under way to identify more genes and markers to develop mulberry varieties tolerant to abiotic stress

through molecular assisted breeding, though till date not much progress could be achieved in this direction. Recently, Jiao et al. (2020) identified several genes from the genome of *M. alba*, among them important are the genes related to the biosynthesis of secondary metabolites and environmental adaptation (e.g., *ABA2*, *CAD9*, and *MPK16*), genes regulating growth and developmental processes and plant yield (e.g., *UBP14*, *SYP124*, and *GATA*). The gene *ABA2* from *M. alba*, involved in the biosynthesis of abscisic acid (ABA), an important plant hormone that affects the synthesis of storage proteins and also confer tolerance to drought and salt stress. Likewise, *GATA* gene involved in regulating chloroplast development and growth in a cytokinin-dependent manner was also detected. Several rapidly evolving genes of *M. notabilis* mainly involved in metabolic pathways and biosynthesis of secondary metabolites were also identified and a few important among them are *FRO2* which undergone rapid evolution and has been positively selected in both *M. alba* and *M. notabilis*. *FRO2* confers low-iron tolerance and also involved in glycine betaine-mediated chilling tolerance and reactive oxygen species accumulation.

7.9.2 Strategies: Landing and Walking

Chromosome 'walking' is a process in which genes are isolated based on genetic maps. The success of isolation of genes by this method is dependent upon the size of the genome and the distance to be "walked" from a known genomic position to the desired gene. It is easy in organisms with small genomes, but is difficult and time consuming in organisms with large size genome. Chromosomal landing on the other identify and isolate clones in a genetic library, thus, it reduces the problems associated with large genomes. In mulberry, to date, no such effort has been made due to the paucity of well saturated genetic maps. Nonetheless, the current efforts to sequence the genome of different species of *Morus* using the next generation sequencing and identification of more marker types, especially those which are abundantly present in the genome like SNPs to going to help develop high density maps in mulberry for positional cloning and analysis of genes and their products.

7.9.3 Libraries

Since large insert genomic DNA libraries are essential for physical mapping, positional cloning, and genome sequencing, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC) and libraries are prepared by fragmenting the genome and cloning the fragments. In BAC fragment size of the range 100–200 kbp size can be inserted into the bacterial chromosome. BAC libraries in general maintain the genome without artifacts or chimerism or rearrangements and are very stable due to the presence of F factors that prevent more than one BAC from simultaneously

inhabiting a bacterium. Thus, BAC libraries are an important resource for the development of molecular markers that can be used for marker-assisted selection (MAS) for desirable agronomic traits. The insert sizes of BAC library are large enough for genomics analysis such as identification of QTLs and construction of physical map of the whole genome. However, to insert bigger size fragment YACs is used which can accommodate fragments of greater than 500 kb. However, some disadvantages of the YAC system include a high degree of chimerism and insert rearrangement that limits its usefulness. Another important vector system useful for map-based cloning of agronomically important genes in plant species is transformation competent artificial chromosome (TAC), which accepts and maintains large genomic DNA fragments stably in both *Escherichia coli* and *Agrobacterium tumefaciens*. This vector is a derivative of P1 phage (Pierce et al. 1992) and contains right- and left-border sequences of T-DNA (Liu et al. 1999), enabling the introduction of cloned DNA into host-plant genomes. The TAC system is useful for not only the positional cloning of genes but also introducing quantitative trait loci (QTLs) covered with TAC contigs. However, in mulberry so far no such libraries have been prepared, and recently some.

7.10 Genomics-Aided Breeding for Resistance/Tolerance Traits

7.10.1 Structural and Functional Genomic Resources Developed

Li et al. (2020a, b, c, d) generated genome-wide, high-coverage DNA methylation maps using whole genome bisulfte sequencing (WGBS) revealing the methylation of individual cytosines in response to drought stress. The methylome map serves as a potential resource for investigating associations between DNA methylation and gene expression and drought stress response, and it provides a means to explore the basis of resistance to aid mulberry breeding. The restriction site associated DNA sequencing analysis (RAD-Seq) was used to elucidate sex determination mechanisms in mulberry (Atsumi et al. 2019). Rukmangada et al. (2020) identified 34,096 unique transcripts, among them 505 transcripts were up-regulated and 597 were down-regulated in the high growth genotypes (HGG). The photosynthetic related genes in HGG are indicative of improved productivity. In addition, they also identified 3893 SSRs, 390,897 SNPs, and 8081 InDels and after validation, they can be used as molecular markers for mulberry improvement.

7.10.2 Details of Genome Sequencing, Assembly and Annotation

The first whole genome sequencing in mulberry was done by He et al. (2013), they used *M. notabilis* with seven distinct pairs of chromosomes (2n = 14) for the genome sequencing. The genome size of *M. notabilis* was found out to be 357.4 Mb and it contains 29,338 genes and 128 Mb repetitive sequences. It is also found that up to ~47% of the mulberry genome is composed of repetitive sequences and nearly 50% of these repetitive sequences were classified into different categories such as *Gypsy*-like (6.58%) and *Copia*-like (6.84%) long-terminal repeat retrotransposons. A total of 27,085 protein-coding loci with complete gene structures was identified high-confidence using 21 Gb RNA-seq data from five tissues. *M. notabilis* was believed to have a minimum 62 chromosome number of 14 and a basic chromosome number of 7 (He et al. 2013). In another effort, Jiao et al. (2020) sequenced the genome of a diploid mulberry species *M. alba* L 28 chromosome (2n = 2x = 28) using combined three different technologies, the Oxford Nanopore, Illumina HiSeq, and high-throughput chromatic conformation capture (Hi-C) platforms and found the genome size as 328.3 Mb. A total of 180.11 Mb of non-redundant repetitive sequences were identified by a combination of de novo and homology-based approaches, which accounted for 52.85% of the assembled genome. A total of 22,767 protein-coding genes were annotated with an average gene length of 3,209 base pairs (bp). The assembled genome of *M. alba* was more complete than the genome of *M. notabilis.* The genomic synteny between *M. alba* and *M. notabilis* showed one-to-one correspondence, where as *Prunus persica* region matched only one *M. alba* region and only one Vitis vinifera region Jiao et al. (2020).

In addition to these, the chloroplast of mulberry has been sequenced (Ravi et al. 2006) using a combination of long PCR and shotgun approaches. The circular double-stranded chloroplast DNA has a size of 158,484 bp comprising two identical inverted repeats of 25,678 bp each, separating by a large and small single copy region of 87,386 bp and 19,742 bp each. From this sequence, 83 protein-coding genes, eight ribosomal RNA genes and 37 tRNA genes were identified. In another attempt, Chen et al. (2016) sequenced the chloroplast of *M. notabilis* and found that the circular genome is of 158,680 bp in size, and comprises a pair of inverted repeat (IR) regions of 25,717 bp each, a large single-copy (LSC) region of 87,470 bp and a small single-copy (SSC) region of 19,776 bp. The chloroplast genome contains 129 genes, including 84 protein-coding genes (PCGs), eight ribosomal RNA (rRNA) genes and 37 transfer RNA (tRNA) genes. The maximum likelihood (ML) phylogenetic analysis revealed that *M. notabilis* was more related to its congeners than to the others. Later, chloroplast sequences from five other species of mulberry were generated (Kong and Yang 2016, 2017).

7.10.3 Impact on Germplasm Characterization and Gene Discovery

The whole genome sequencing of mulberry had impacted significantly the characterization of germplasm as illustrated with 134 mulberry accessions by Jiao et al. (2020). Using the newly identified 14,273,912 high-quality SNPs, the phylogenetic relationship among 132 cultivars using 2 wild mulberry genotypes was worked out and it was found that the phylogenetic tree generated with these SNPs was not inconsistent with the traditional species delimitations. Cultivars from Chinese origin were grouped into two viz., Hu mulberry (HU), from Taihu Basin, and non-Hu mulberry (NH), from the rest of China. This latter group is further divided into two subgroups, East and West. A lower level of heterozygosity with high linkage disequilibrium decay was also observed. Likewise, using genome-wide 2229 SNPs Muhonja et al. (2020) worked out the genetic relationship among 54 mulberry accessions from seven species (*M. alba, M. indica, M. bombycis, M. acidosa, M. latifolia, M. kagayamae*, and *M. rotundiloba*). The phylogenetic tree had only three clear monophyletic clades viz, two Japanese native species, *M. acidosa* and *M. kagayamae* from different geographically isolated islands and a Thai species, *M. rotundiloba*, and all other species were found non-monophyletic. It was also observed that no distinct monophyletic clades were formed by varieties from M. *alba* and *M. latifolia,* indicating the formation of hybrids through natural cross hybridizations. These studies suggested that the present classifications of species under the genus *Morus* need revision but it may not be an easy task even with genome-wide DNA markers. Earlier studies with inter-simple sequence repeat (ISSR) and ITS markers also supported such views (Muhonja et al. 2020; Zhao 2005).

7.10.4 Genetic Enhancement of Indian Mulberry

Genetic enhancement is defined as transferring useful genes from exotic or wild types into agronomically acceptable background (Jones 1983). Genetic enhancement is also termed as pre-breeding or developmental breeding (Rick 1984) used the term "genetic enhancement" or "pre-breeding" to describe the activity of transferring or introgressing genes or gene combinations from unadapted sources into breeding materials. Genetic enhancement and traditional breeding for cultivar development differ from each other with the objectives. Genetic enhancement aims at improving the germplasm to use for breeding traditional breeding to develop cultivars. Thus, pre-breeding is the process of identifying useful traits from wild species and relatives and transferring them into accessions that can be used for cultivar development. In mulberry, not much effort has been made in this direction. Nonetheles, Tikader and Dandin (2007) used prebreeding program to incorporate useful traits from wild species to domestic species by crossing *M. alba* with *M. laevigata* and *M. serrata.* The F1 hybrids obtained from these crosses exhibited significant heterosis for single

leaf weight, leaf area, leaf yield and rooting of the stem cuttings also. Based on the observations made on these hybrids it was concluded that introgression of useful traits from wild species to the domesticated could be possible even in the F1 generation (Tikader and Dandin 2008). Since, genetic enhancement through traditional means is laborious, time taking and much resource consuming, efforts were initiated recently to use molecular and biotechnological means. Pavan (2010) successfully carried out isolation and fusion of protoplasts from *M. indica* cv. S13 with *M. indica* cv. S36 using electric fusion process. Mesophyll derived protoplasts of S13 and S36 were pipetted into an electrofusion chamber in 1:1 ratio. Differentiation of protoplasts of two cultivar types was made based on the size of protoplasts, hence, during fusion, the small sized protoplasts of S36 type got fused with large sized protoplasts of S13 (Pavan 2010). The regenerated plants have been transferred to germplasm for detailed evaluation and utilization in the breeding program.

7.11 Recent Concepts and Strategies Developed

7.11.1 Gene Editing

Abiotic stress is a complex trait controlled by a number of genes and their products that are involved in signaling, regulatory and metabolic pathways, thus, just a modification in a single gene may not produce any desired results. Therefore, more advanced and effective techniques that affect several genes simultaneously need to be applied. Gene manipulation with CRISPR-Cas 9 is one such technique. CRISPR (clustered regularly interspaced short palindromic repeats) and CRISPR-associated (Cas) genes are an essential component of a bacterial adaptive immune system to acquire resistance against invading virus. CRISPR/Cas9 uses a protein-RNA complex to target and cleavage the target sequence using a short guide RNA (Pennisi 2013). Scientists have found great many utility for this system in gene manipulation such as the introduction of single point mutations, deletions, insertions, inversions, translocations etc. in a particular target gene. In general, gene manipulation of monogenic trait is always easier than those controlled by many genes. However, abiotic stress is a trait controlled by a number of genes involved in gene regulations, signaling and in several metabolic pathways. Thus, simultaneous manipulation of many of these genes is required to get the desired results. CRISPER-Case 9 can target several genes simultaneously due to the easiness of designing high efficiency sgRNAs. Multiplex genome editing has been successfully implemented in model crop plants (Li et al. 2013; Mao et al. 2013; Zhou et al. 2014). It is also possible to create mutations as sgRNA libraries targeting almost all the genes can be generated to induce genome-wide point mutations and gene knock-outs. The screening of mutants with altered abiotic stress response in plants could enable gene function analyses and the generation of stress-tolerant crop varieties. Further, the use of CRISPR-based synthetic

transcriptional activator or repressor to modulate the transcription of target endogenous genes has already been demonstrated in plants (Piatek et al. 2015). The use of CRISPR-Cas9 system in genotyping natural variations to distinguish homozygous biallelic mutants from wild-type has been demonstrated (Kim et al. 2014). However, in mulberry CRISPER based technology has not been used yet. Thus, with the advancement of the genomics of mulberry, it is expected that gene editing with CRISPR-Cas9 technology would be applied in mulberry soon.

7.11.2 Nanotechnology

Nanotechnology is the art and science of manipulating and rearranging individual atoms and molecules to create useful materials, devices, and systems. Nanotechnology has a lot of applications in agriculture such as crop improvement, precision farming, plant disease monitoring, soil remediation, removal of heavy metals, water treatment, nano fertilizer, nano-pesticide, artificial intelligence for automated farming activities, identification of pest and diseases, managing crop quality, monitoring biotic and abiotic stresses. Hydrogels, nanoclays, and nanozeolites have been reported to enhance the water-holding capacity of soil (Sekhon 2014) and nanotubes and nano metals and metal oxides have been used for soil remediation as they have the capacity to absorb soil and water contaminants (Khin et al. 2012). Nanotechnology has been used in gene therapy for plants as 3-nm mesoporous silica nanoparticle (MSN) can introduce foreign DNA into cells, DNA sequencing using a fluidic nanochannel functionalized with a graphene nanoribbon, increases the germination through better penetration of the moisture. Nanobio-sensors have been utilized to increase sensitivity to monitor soil conditions and crop growth over vast areas. Nano fertilizers are also used for increasing in Nutrient Use Efficiency (NUE). In mulberry, baring a few isolated efforts to use hydrogels very little application has been done so far.

7.12 Brief on Genetic Engineering for Resistance/Tolerance Traits

Genetic engineering consists of the isolation of a gene of interest, ligating it on a vector to transfer it into a plant genome to meet a purpose. The most important advantage of genetic engineering is the ability to manipulate gene expression as desired. In plant breeding, the breeders can work only with plants that are cross-fertile but with genetic engineering genes from any organism including micro organisms can be inserted into the plant. However, the biggest challenges are the development of robust, reproducible plant regeneration protocol and a genetic transformation method. In mulberry, such an efficient protocol for direct plant regeneration from leaf explants

is still to be developed, though direct plant regeneration from hypocotyls has become an easy task (Vijayan et al. 2011b).

7.12.1 Target Traits and Alien Genes

In mulberry, the main trait that targeted much is the leaf yield, which is the product of cumulative contributions from a number of associated traits such as plant height, leaf weight, number of branches, leaf retention capacity, nodal length, root length (Vijayan et al. 1997a). However, under saline conditions a change in correlation was observed as the leaf yield had significant correlation with plant height, leaf size, shoot weight, root weight, root length, protein, NRase activity and WUE of the plant. Similarly, the plant height was also found changing its correlation with most of the characters studied. This clearly shows that under different salinity levels the selection criteria for plants needs to be adjusted (Vijayan et al. 2009a). It has also been observed that mulberry possess certain traits to confer higher tolerance to stress conditions. Some of these traits are thicker epicuticular wax, elongated roots, synthesis and accumulation of osmolytes like proline, glycine beataine, etc. (Vijayan et al. 2008a, b). Plants have evolved several mechanisms like paraheliotropic movements, thicker epicuticular wax, elongated roots, salt-secreting hairs, synthesis and accumulation of osmolytes, etc. to tolerate the stress to facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis (Vijayan et al. 2008a). The genes involved in these pathways and mechanisms need to be incorporated either through conventional breeding or through modern techniques like genetic engineering. Since, transgression of genes and traits into mulberry is very difficult due to the breeding behavior of the plant the easiest method is through genetic engineering. In genetic engineering, genes may be knocked out, overexpressed, or modified through gene editing. Over expression of *DREB*s (dehydration responsive elements binding proteins), MYB (myeloblastosis), *ERF* (Ets-2 Repressor Factor), *bZIP*, and *WRKY* transcription factor families has showed promising results in several plant species (Jung et al. 2007). Further, Lu et al. (2008), have identified a low-temperature induced gene *WAP25 f* rom mongolian mulberry, one of the wild species of genus *Morus* that grows in cold regions and cloned the gene (GenBank accession N0. DQ104333) into expression vector pIG121/*Wap*25 and transformed *Petunia hybrida* Vilm via *Agrobacterium.* This study exhibited the possibility of genetic improvement of other mulberry species such as *M. alba, M. indica, M. latifolia* which are being used for silkworm rearing and are highly susceptible to cold and other stresses.

7.12.2 Review on Achievements of Transgenics

The first attempt to introduce a foreign gene through *A. tumefaciens*-mediated transformation in mulberry was made by Machii (1990). Later, Oka and Tiwary (2000) induced hairy roots at the base of hypocotyls by infecting with *Agrobacterium rhizogenes.* Subsequently, Nozue et al. (2000) transformed calli initiated from cotyledons, hypocotyls and roots of seedlings co-cultivated with *A. tumefaciens.* Subsequently, using *A. tumefciens*, transgenic mulberry plants containing the glycinin gene *AlaBlb*, the oryzacystatin gene *OC* (Wang et al. 2003), and the barley *HVA1* gene (Lal et al. 2008) were developed. The transgenic mulberry plants overexpressing *HVA1*, a group-3 LEA protein isolated and characterized from barley, showed increased cell membrane stability, higher relative water use efficiency and growth under salt stress (200 mM NaCl) in mulberry (Lal et al. 2008). Physiological, biochemical and molecular studies revealed that this transgenic mulberry plant performed much better than the non-transgenic plants when subjected to salinity (200 mM NaCl) and drought (2% PEG, MW 6,000) induced stresses. Transgenic plants showed better cell membrane stability, photosynthetic yield, less photo-oxidative damage, and high relative water content, under salinity and water stress. Das et al. (2011) developed a transgenic mulberry overexpressing a tobacco osmotin gene using both CaMV35S promoter and a stress-inducible promoter *rd29A*. The *rd29A* (responsive to dessication) promoter from *Arabidopsis* with two major cis-acting elements, the abscisic acid (ABA)-responsive element (ABRE) and the dehydration-responsive elements (DRE) responded well to drought stress. It has also been observed that transgenic plants with the stress-inducible promoter had better salt tolerance than those with the constitutive promoter. Transgenic *M. indica* plant overexpression of beta-carotene hydroxylase1 (BCH1) found to be more tolerant to salt and temperatures due to better membrane integrity under stress conditions (Saeed et al. 2015).

7.12.3 Organelle Transformation

In plants the most widely manipulated cell organelle is the chloroplast as chloroplast transformation has multiple advantages over nuclear genome engineering. In chloroplast transformation, transgene is integrated via homologous recombination, thus, eliminating gene silencing and position effect. Further, the chances of spreading the transgene genes through pollen transmission are also very minimal. Although in mulberry no such plants has been developed, chloroplast engineering has successfully employed in several other plant species to confer enhanced resistance to stresses through simultaneous expression of protease inhibitors and chitinase (Chen et al. 2014), herbicide resistance, insect resistance (Jana 2010). Since the sequencing of the mulberry chloroplast genome has already been completed (Chen et al. 2016); identification of endogenous regulatory sequences for optimal transgene expression

is easier than before. Further, different gene delivery systems like particle bombardment, *Agrobacterium* assisted gene transfer along with robust selection and regeneration protocols for transplastomic cells have already been developed in mulberry. It is also pertinent to note that it would be easier to get regulatory approval for chloroplast transformed plants than nuclear transformed. Thus, in the coming days more focus will be on chloroplast genome engineering in mulberry for the development of transplastomic mulberry with improved agronomic traits and tolerance to abiotic stresses.

7.12.4 Biosynthesis and Biotransformation

Bioinformatics is increasingly becoming an indispensable tool for genomics research due to its untapped potential to extract information from nucleotide sequences of various types. Whether it is whole genome sequence or transcriptome, bioinformatics is essential to analyse them properly to draw inferences. Although bioinformatics and genomics have been developed well in many other crops, the same has not been developed well in mulberry. Baring a few resources like MorusDB (http://morus.swu.edu.cn/morusdb) providing information on genomic data and EST of *M. notabilis*; (http://btismysore.in/mulsatdb/) (Krishnan et al. 2014b; Li et al. 2014) not much information is available on this aspect.

7.12.5 Metabolic Engineering Pathways and Gene Discovery

Since leaf is the primary product for which mulberry is cultivated and more than 70% of the mulberry leaf protein is used by the silkworm for synthesizing the silk yarns, the nutritional status of mulberry leaf plays a crucial role in deciding the suitability of the variety for silkworm rearing (Fukuda et al. 1959). However, in mulberry, not much work has been done to develop metabolic profiles of the varieties and their relation with silk productivity. Nonetheless, studies in other crops have demonstrated the importance of such studies to understand the metabolic networks and the underlying mechanisms of interaction between metabolism and development (Fernie and Schauer 2008). The most commonly used technologies for metabolic profiling are mass spectrometry and nuclear magnetic resonance (NMR). Once the metabolic profiling and underlying genetic interactions are unraveled, it would be easy to engineer plants with desired metabolites. Metabolic engineering has been successful in the development of golden rice and various transgenic crops fortified with flavonoids, anthocyanin, carotenoids, Omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), vitamins and minerals (Storozhenko et al. 2007). In mulberry, screening of glucosidase inhibitors from leaves found glucosidase inhibitory activities in all the constituents of alkaloids, flavones and amylases (Yuan et al. 2006). These alkaloidal sugar-mimic glycosidase inhibitors present in higher concentrations in mulberry

latex (1,4-dideoxy-1,4-imino-D-arabinitol, 1-deoxynojirimycin, and 1,4-dideoxy-1,4-imino-D-ribitol). Realizing the importance of chemical contents of the leaves and fruits of mulberry, anthocyanin composition, 1-deoxynojirimycin (DNJ), resveratrol, oxyresveratrol, and flavanoids estimated and found significant variations in concentrations of these compounds in different species and cultivars (Bae and Suh 2007; Song et al. 2009). Ni et al. (2010) isolated cathayanons F-J and cathayanin A, and two known compounds, cathayanins B-C from the stem bark of *Morus cathayana.* Recently, Li et al. (2020a) has assessed the flavones in mulberry and identified a Fe2+/2-oxoglutarate-dependent dioxygenase from *M. notabilis* (MnFNSI) showing the typical enzymatic activity of a FNSI-type enzyme, and directly converts eriodictyol and naringenin into their corresponding flavones. Overexpression of MnFNSI in tobacco increased the flavones contents in leaves and enhanced the tolerance of tobacco to ultraviolet-B (UV-B) stress. Later, Li et al. (2020b) also made the metabolic profiling of 44 flavonoid compounds from 91 mulberry resources and found that O-rhamnosylated flavonols and malonylated flavonol glycosides, including rutin and quercetin 3-O-(6″-Omalonylglucoside) (Q3MG), were absent from *M. notabilis* and multiple mulberry (*M. alba* L.) resources. Transcriptome and phylogenetic analysis of flavonoid-related UDP-glycosyl transferases (UGTs) suggested that the flavonol 3-O-glucoside-Orhamnosyltransferase (FGRT) KT324624 is a key enzyme involved in rutin synthesis. A recombinant FGRT protein was able to convert kaempferol/quercetin 3-O-glucoside to kaempferol 3-O-rutinoside (K3G6″Rha) and rutin. The recombinant FGRT was able to use 3-O-glucosylated flavonols but not flavonoid aglycones or 7-O-glycosylated flavonoids as substrates. The enzyme preferentially used UDP-rhamnose as the sugar donor, indicating that it was a flavonol 3-O-glucoside: 6″-O-rhamnosyltransferase. The above studies would certainly augur further research in mulberry to engineer the metabolic pathways through genetic engineering for developing mulberry varieties with more nutritious leaf, stress tolerant and silkworm friendly.

7.12.6 Gene Stacking

Developing crop varieties that can withstand incidence of multiple stresses is one of the major objectives of crop breeding. To achieve the same, it may require bringing multiple genes from different sources into a single plant and the process is called gene stacking or gene pyramiding. Gene pyramiding is more effective in developing plants with improved agronomic trait/stress tolerance/resistance/metabolic traits than single trait introduction. Different techniques have been used to achieve the target. One of the commonly used techniques is to identify quantitative trait loci (QTL) of the traits and introgress them into a single plant through marker assisted selection breeding. Another method is the generation of transgenic plants by introducing two or three foreign genes is one of the common means to develop plants exhibiting resistance against multiple stresses. Co-transformation is one the most promising approaches for introduction of multiple genes into plants. Co-transformation could apply to

several plant species and with both direct transformation using physical agents and indirect transformation with *Agrobacterium* mediated transformation through co-culturing of plant tissue with *Agrobacterium* harboring multiple gens in T-DNAs. So far, gene stacking method has not been reported in mulberry. Nonetheless, the recent developments in genomics and transgenesis will help develop multiple stress tolerant mulberry plants by stacking several traits/genes through transgenesis and marker assisted selection breeding.

7.12.7 Gene Silencing

Gene silencing (GS) is defined as a molecular process involved in the down regulation of specific genes, and probably evolved as a genetic defense system against viruses and invading nucleic acids (Wassenegger 2002). Different techniques involving post-transcriptional gene silencing or RNA interference (PTGS or RNAi) (Vaucheret et al. 2001), transcriptional gene silencing (TGS) (Vaucheret and Fagard 2001), microRNA silencing (Bartel 2004), and virus induced gene silencing (VIGS) (Burch-Smith et al. 2004) have been used to manipulate expressions of endogenous genes and invading nucleic acids in plants. RNAi is achieved through the expression of a hairpin RNA (hpRNA) that folds backs to create a dsRNA and gives rise to siRNAs to effect gene silencing (Wesley et al. 2001). Blocking the gene expression through DNA methylation of coding and promoters regions and consequent chromatin remodeling is called transcriptional gene silencing (TGS) as DNA methylation promotes protein binding on the methylated cytosine to remodel the chromatins to prevent binding of transcription factors resulting in transcriptional gene silencing (Alberts et al. 2002; Lippman and Martienssen 2004). MicroRNAs (miRNAs) are ~22 nt endogenous RNAs present abundantly in multicellular organism capable of cleavage or repressing translational of mRNA and constitute a substantial amount of the gene regulatory molecules in plants (Duan et al. 2005). miRNAs are known to be involved in regulating mechanisms for stress and environmental responses (Bonnet et al. 2006). In mulberry baring, a few reports not much work has been done on gene silencing. Li et al. (2018b) demonstrated that silencing of *MmSK* gene, which codes a Shaggy-like protein kinase, results in higher sensitivity to drought stress in mulberry.

7.12.8 Prospects of Cisgenics

Cisgenesis is the genetic modification of a recipient plant with a natural gene from a sexually compatible plant and it involves only genes from the plant itself or from a close relative with a single step gene transfer mitigating the risks associated with alien gene introgression. Cisgenic plants can harbor one or more cisgenes, but they do not contain any transgenes (Hou et al. 2014). In the case of a cisgenic plant, the gene of interest, together with its promoter, is present in the species or in a

sexually compatible relative for centuries. Therefore, cisgenesis does not alter the gene pool of the recipient species and provides no additional traits (Schouten et al. 2006). Similarly, unlike transgenesis, cisgenesis does not pose any deleterious effect on the non-target organisms or environment as the gene transfer would be much like that of traditional breeding. However, cisgenesis has great potential to overcome the major bottlenecks like linkage drag, sexual incompatibility etc. that impede the traditional breeding as in cisgenesis only the gene of interest is isolated from the donor plant and inserted into the recipient plant. Hence, cisgenesis is very useful for outcrossing and highly heterozygous plants like mulberry with long generation gaps. In mulberry so far only a few reports towards cisgenesis are available. Saeed et al. (2015) incorporated β-carotene hydroxylase1 (bch1) in mulberry cultivar K2 through *Agrobacterium* mediated genetic transformation and found the enhanced level of carotenoids and tolerance to high light, heat and UV irradiation. Fang et al. (2019) identified a *MuPR1* genes and its expression increased resistance to diseases. Nevertheless, the recent progress in mulberry genome sequencing would expedite the identification and isolation of genes of interest from the gene pool to develop more cisgenic plants.

7.13 Brief Account on Role of Bioinformatics as a Tool

7.13.1 Gene and Genome Databases

Bioinformatics has been used extensively in mulberry to identify genes, predict their functions and also develop molecular marker systems for germplasm characterization and genetic diversity assessment. In order to find out gene sequences and molecular markers, the whole genome (He et al. 2013; Jiao et al. 2020) has been sequenced and analysed. In addition, Ravi et al. (2006) sequenced the chloroplast of an Indian mulberry cultivar (*M. indica* cv. K2) and found that out of the total 158,484 bp circular double-stranded DNA, two identical inverted repeats of 25,678 bp each, separating a large and a small single-copy region of 87,386 bp and 19,742 bp, respectively were observed with 83 protein-coding genes including five genes duplicated in the inverted repeat regions, eight ribosomal RNA genes and 37 t RNA genes (30 gene species) representing 20 amino acids. Using this information, it inferred that *Morus* is closer to *Cucumis* and *Lotus* and Rosales closer to Fabales phylogenetically (Ravi et al. 2007). Later chloroplast sequencing has been done in *M. notabilis* (Chen et al. 2016), *M. mongolica* (Kong and Yang 2016), *M. atropurpurea* and *M. multicaulis* (Li et al. 2016), *M. cathayana* and *M. multicaulis* (Kong and Yang 2017). These studies showed only considerable variations in the length and number of predicted genes. The gene sequences and data of the above findings are available on websites like NCBI, MorusDB, MulSat, https://ddbj.nig.ac.jp/DRASearch/query?organism=Morus%20alba.

7.13.2 Gene Expression Databases

Data on expressed sequence tags (ESTs), suppression subtractive hybridization (SSH) and global transcriptome are valuable resources for genetic improvement of crops. In mulberry, quite a few efforts have been made to analyse transcriptomes from different species, conditions and plant parts (Lal et al. 2009; Checker et al. 2012; Wang et al. 2014a, 2018; Huang et al. 2020). A total of 958 and 1878 expressed sequence tags (ESTs), respectively were identified from mulberry leaf and root cDNA libraries (Lal et al. 2009; Checker et al. 2012). Approximately 1500 ESTs were identified from water-stressed leaves using suppression subtractive hybridization (Gulyani and Khurana 2011). Wang et al. (2014a) used de novo assembly of 54,736 contigs to identify 247 pathways and thousands of SSR markers. Seven unique genes showing different expression levels in control and drought stress groups were also identified with real-time PCR. Likewise to understand molecular mechanism on anthocyanin biosynthesis in mulberry fruit, comparative transcriptome analyses of *M.atropurpurea* Roxb, (black fruit) and *Morus alba* L., (white fruit) were made using more than 27,085 genes (including 1,735 new genes) differentially expressed at three developmental stages and found that anthocyanin biosynthesis is associated with the expression of 15 core genes and 5 transcription factors (Huang et al. 2020). Similarly, to unravel the genomic response of mulberry to stress conditions, 101,589 unigenes obtained from 24 mulberry accessions under salinity were analysed and found that the number of down-regulated DEGs (differentially expressed genes) under salt stress is more than that of up-regulated DEGs, and these down-regulated DEGs enriched in the process related to stress responses. Further, it has been observed that the expression pattern of some genes was quite diverse against salt stress and it was genotype- and tissue-dependent. The DEGs involved in signal transduction and transcription regulation were more in low-salt-tolerant genotypes and the majority of these responsive genes showed a reduction in the transcripts (Liu et al. 2017). In another attempt, Wang et al. (2018) assessed two cDNA libraries of leaf from mulberry genotypes with different 1-deoxynojirimycin contents and found that 11,318 transcripts significantly and differentially expressed, and 38 unique genes were identified involved in DNJ alkaloid biosynthesis in mulberry. Li et al. (2020b) made a transcriptome analysis of flavonoid-related UDP-glycosyltransferases (UGTs). Further, to understand the role of microRNAs (miRNAs) in gene expression under drought stress, transcriptome-wide high throughput degradome sequencing was used and identified 409 target genes of 30 conserved miRNA families and 990 target genes of 199 novel miRNAs. Of the conserved miRNA families, mno-miR156, mno-miR172, and mno-miR396 had the highest number of targets with 54, 52 and 41 transcripts each signifying that these three miRNA families might play key roles in drought responses in mulberry (Li et al. 2017). Similarly, it was also discovered that mulberry has 286,122 MITE-related sequences, including 90,789 full-length elements (Xin et al. 2019). Using transcriptome sequencing technology Jiang et al. (2020) investigated the molecular mechanisms of mulberry response to Cadmium (Cd) stress. They obtained 195 million clean reads, 2785 and 1211 differentially expressed genes (DEGs) enriched

in the pathway of flavonoid biosynthesis, plant pathogen interaction, carbon fixation in photosynthetic organisms and phenylpropanoid biosynthesis. The flavonoids under Cd stress could be the effective antioxidants conserved in mulberry.

7.13.3 Protein or Metabolome Databases

In order to provide a specialized metabolic database for mulberry Li et al. (2020c) developed the MMHub (https://biodb.swu.edu.cn/mmdb/). It is a user-friendly, freely available and comprehensive metabolomics database from 91 mulberry resources. The investigators are committed to continuously updating this database with strictly curated data, including more metabolites and more tissues. MMHub has several features and advantages like (i) all metabolomics information, including metabolites, structure, mass spectra and validated metabolite concentrations, are strictly curated and verified; (ii) as an initial metabolomics data repository for mulberry, it can be used to provide basic data for other databases; and (iii) It will be updated regularly with more data on metabolomics of mulberry (iv) queries from other researchers can be submitted for analysis and comparison.

7.13.4 Integration of Different Data

In order to understand the molecular mechanisms on stress tolerance in mulberry, an integrated physiological and TMT (tandem mass tag)-based proteomics analysis was carried out on mulberry by Liu et al. (2019) using 30 seedlings after subjecting to two weeks of combined salt and drought stress. The study revealed that 343 upregulated proteins and 234 downregulated proteins in leaves, 181 upregulated proteins and 89 downregulated proteins in roots after the stress treatment, and 50 proteins expressed commonly during the process of salt-drought stress. It was also observed that, out of the 104 different metabolic pathways, the top three from leaf were "carbon metabolism", "ribosome", and "starch and sucrose metabolism", and the top three from root were "starch and sucrose metabolism", "phenylpropanoid biosynthesis" and "pentose and glucuronate interconversions. To understand the reason for the feed specificity of silkworm to mulberry leaf, an integrated study was carried out by Wang et al. (2017a) and found that out of the 2076 foliar proteins, 210 were found in the silkworm feces. The chloroplast proteins accounted for 68.3% in mulberry leaves and 23.2% in the feces and two key proteins responsible for synthesizing jasmonic acid were also observed. Although the amount of secretory-pathway proteins was low in mulberry leaves (7.3%) but was more in the feces (60.1%). The comparative proteomics analyses indicated that mulberry leaves not only provide amino acids to the silkworm but also display defense against silkworm feeding.

7.14 Brief Account on Social, Political and Regulatory Issues

7.14.1 Concerns and Compliances

Although much has been achieved in the field of genetic manipulations, taking the fruits of all these efforts needs strong political and regulatory intervention as in many countries especially in Asian countries the genetically modified organisms (GMO) have not been approved and accepted well. Thus, it is time for focusing more on these aspects as along with the developments in the field of genomes and genetics, it is also necessary to make efforts to change the mindsets of the public so that the modern biotechnological tools CRISPER/Cas can be used more commonly to address several issues like tackling of severe stress conditions to make the agriculture based sericulture more sustainable.

7.14.2 Patent and IPR Issues

Intellectual property rights (IPRs) are the rights given to persons for a certain period of time over the creations they made. The term "intellectual property" was first used by Massachusetts Circuit Court ruling in a case *Davoll et al Vs Brown* in October 1845. Different categories of IPRs such as breeders' rights, patent rights, trade secrets, geographical indications and trade marks in relation to agriculture, thereby providing an insight into various facets as relevant to agricultural research and development. Development of a new plant cultivar or variety, either by "traditional" breeding methods or by "modern" molecular modification, requires a lot of time and effort. To recover the costs of this research and development, the breeder may seek to obtain exclusive marketing rights for the new variety. Plant variety protection is a good choice for many breeders. International Convention for Protection of New Varieties of Plants (UPOV) created the *sui generis* system for protection of new varieties of plants "(GATT Secretariat, 1994, The Results of the Uruguay round of Multilateral Trade Negotiations: The Legal Texts, Switzerland)". The *sui generis* system is a milder/diluted form of a patent and it accords the legislation of plant variety protection through which breeders, researchers and farmers rights are protected, Business Guide to Uruguay Round, 1995 (Anonymous 1995b). The Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) is the government agency that handles plant variety protection issues in India as the registration under the Protection of Plant Varieties and Farmers" Rights (PPV & FRA) Act, 2001. In order to register new mulberry varieties under the PPV & FRA rules, the variety is subjected to the Distinctiveness, Uniformity, and Stability (DUS) test wherein a set of characters identified by the authority is evaluated. The details of

which can be obtained from http://www.csrtimys.res.in/sites/default/files/menufiles/DUS-Guidelines.pdf. Another form of IPR is patents that provide protection for patentable plants and animals and biotechnological processes by giving the patentee the right to prevent third parties from making, using, or selling the patented product or process. Patents are generally disclosed to the public through patent documents to enable the researchers to develop further useful products or services. In order to patent a finding, it should meet certain criteria of patentability like novelty, non-obviousness, etc. depending on the law of the land. The geographical indications are marks associated with the origin of products such as country, region or locality where the quality, reputation or other characteristics of the product are essentially attributable to its geographical origin. No mulberry varieties were patented or given any geographic indications.

7.14.3 Disclosure of Sources of GRs, Access and Benefit Sharing

The mulberry genetic resources (GRs) consists of diverse types of collections such as primitive cultivars, natural hybrids, wild and weedy relatives, wild species, obsolete varieties, elite lines, breeding lines, mutants and polyploids, interspecific and intergeneric hybrids developed systematically (Vijayan et al. 2005). Genetic resources are much important for developing new varieties as it contains genes for traits of interest. Keeping this in view efforts have been made by several countries to collect and conserve GR from different countries. However, over the years several regulations have been developed by different countries to protect biodiversity, to ensure fair and equitable sharing of benefits arising out of the utilization of genetic resources. 'Convention on Biological Diversity' (CBD) of 1992 and the recent Nagoya protocol, a 2010 supplementary agreement to the CBD, served as the foundation for the regulations for many countries. Countries introduced disclosure requirements (DRs) containing (i) the GR origin and/or source used in the invention, (ii) evidence of prior informed consent (PIC) on GR access, and (iii) evidence of a benefit-sharing agreement (MAT). Mulberry GR have been exchanged among the sericultural important Asian countries for wide hybridization programme and other research purposes.

7.15 Future Perspectives

7.15.1 Potential for Expansion of Productivity

Research during the last couple of decades has enabled to enhance the mulberry leaf productivity from the 30 to 60 MT/Ha/Yr. However, one of the most important aspects that received least consideration is the leaf quality and also to develop varieties that

can be cultivated in marginal lands as many of the mulberry cultivating areas are being converted into residential and industrial areas. Since mulberry is a tree crop being propagated through vegetative means, once a gene for stress tolerance is incorporated either through conventional breeding or through the modern biotechnological techniques in high yielding plants, the same can be perpetuated without any modifications. This property of mulberry has given an extra advantage to the researchers. However, due to the long gestation period, lack of repeatable and precise screening techniques, little progress could be made on the abiotic stress tolerance research in mulberry. Since stress tolerance is a polygenic trait, pyramiding of a number of relevant traits in a single genotype is often required. Thus, a multidisciplinary approach involving genetics, biochemistry, biotechnology, physiology, and plant breeding is required to develop stress tolerant varieties in mulberry. Likewise, to utilize the natural variability available within the genetic resources of mulberry, an extensive characterization programme should be undertaken utilizing all the germplasm accessions to identify genotypes with better tolerance to stresses for breeding programmes. The current pace of advancements in biotechnology and molecular biology would take abiotic stress research in mulberry to a new height where tailor made varieties could be developed to enhance mulberry leaf productivity both horizontally and vertically.

7.15.2 Potential for Expansion into Nontraditional Areas

The global population explosion, urbanization, salinization of arable lands and global warming are the major challenges world agriculture is going to face in the coming days. Sericulture, being an agro-based industry, has to face these challenges along with competition from other agriculture crops. Although most of the sericultural important countries have come up with high yielding varieties, expansion of mulberry cultivation into nontraditional areas like resource constrained marginal lands, suboptimal conditions, lands and soil-stressed conditions such as drought and salinity like abiotic stress affected areas continues to be a problem yet to be resolved. Soil salinity already affects more than 6% of the total global area and is increasing daily due to intensive irrigation practices (Flowers and Yeo 1995). The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed (Yokoi et al. 2002). In India, nearly 142 million ha of land are affected by drought (http://www.gisdevelopment.net) and about 7.3 million ha of arable land are affected by salinity and alkalinity (http://www.cssri.org). Thus, there is a great potential for expansion of mulberry cultivation into these lands if suitable mulberry varieties are developed, which would be possible with the help the modern molecular tools.

References

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

Abdurakhmonov IY, Kohel RJ, Yu JZ, Pepper AE, Abdullaev AA, Kushanov FN, Salakhutdinov IB, Buriev ZT, Saha S, Scheffler BE, Jenkins JN (2008) Molecular diversity and association mapping of fiber quality traits in exotic *G. hirsutum* L. germplasm. Genomics 92(6):478–487

Agarwal RR, Yadav JSP, Gupta RN (1982) Saline soils of India. Indian Council of Agricultural Research, New Delhi

Aggarwal RK, Udaykumar D, Hender PS, Sarkar A, Singh L (2004) Isolation and characterization of six novel microsatellite markers for mulberry (*Morus indica*). Mol Ecol Notes 4(3):477–479

Ahanger M, Ramegowda G, Illahi I, Rizvi G, Dhar A, Sahaf K (2013) Reaction of mulberry germplasm to frost damage in Kashmir. Res J Agri Sci 4:180–183

Ahmed F, Sultana R, Ahmed O, Akhtaruzzaman M, Iqbal MT (2017) Roles of different fertilizer management practices on mulberry leaf yield and quality. Intl J Agri Biol Eng 10(5):104–114

Akio K, Hiroaki Y, Hiroaki M (2002) Conservation status of mulberry genetic resources in Japan. Paper contributed to Expert consultation on promotion of global exchange of sericulture germplasm satellite session of XIXth ISC congress, Sept 21–25, 2002, Bangkok, Thailand, pp 1–7

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002) Molecular biology of the cell, 4th edn. Garland Science, New York

Allem AC, Mendes RA, Salomão AN, Burle ML (2001) The primary gene pool of cassava (*Manihot esculenta* Crantz subspecies esculenta, Euphorbiaceae). Euphytica 120(1):127–132

Ananthakrishna KH, Sarpeshkar AM, Muralidhara HR (1995) Drip irrigation for mulberry cultivation. Indian Silk 34(3):17–19

Anil Kumar HV, Muralidhar TS, Munirajappa R (2012) RAPD analysis of EMS mutagenized mulberry genotype RFS135. Schol J Biotechnol 1:1–7

Anil Kumar HV, Muralidhar TS, Acharya S, Das MJ (2013) EMS induced morphometric biomass and phytochemical variations in Morus species (Genotype RFS135). J Exp Agri Intl 3(1):43–55

Anonymous (1995a) Mulberry breeding manual. Sericulture Department, Zhejiang Agricultural University, Hangzhou

Anonymous (1995b) Secretariat, C. and Centre du commerce international CNUCED/OMC, Business guide to the Uruguay round. Commonwealth Secretariat, p 26

Anuradha JH, Vijayan K, Nair CV, Manjula A (2013) A novel and efficient protocol for the isolation of genomic DNA from mulberry (*Morus* L.). Emir J Food Agri 25:124–131

Arfan M, Khan R, Rybarczyk A, Amarowicz R (2012) Antioxidant activity of mulberry fruit extracts. Intl J Mol Sci 13(2):2472–2480

Arora V, Ghosh MK, Gangopadhyay G (2014) SSR markers for assessing the hybrid nature of two high yielding mulberry varieties. Intl J Genet Eng Biotechnol 52:191–196

Arora V, Ghosh MK, Ghosh PK, Kumar SN, Gangopadhyay G (2015) Molecular markers to assess the hybrid nature of an improved mulberry genotype. J Plant Sci Res 2(1):116

Arunakumar GS, Gnanesh BN, Manojkumar HB, Gandhi DS, Thallapally M, Sivaprasad V, Tewary P (2021) Genetic diversity, identification and utilization of novel genetic resources for resistance to *Meloidogyne incognita* in Mulberry (*Morus* spp.). Plant Dis 31(ja)

Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kato A, Nash RJ, Lee HS, Ryu KS (2001) Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). J Agri Food Chem 49(9):4208–4213

Ashraf M (1994) Breeding for salinity tolerance in plants. Crit Rev Plant Sci 13(1):17–42

Ashraf M, Karin F, Rasul E (2002) Interactive effects of gibberellic acid (GA 3) and salt stress on growth, ion accumulation and photosynthetic capacity of two spring wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. Plant Growth Regul 36(1):49–59

Atsumi R, Nishihara R, Tarora K, Urasaki N, Matsumura H (2019) Identification of dominant genetic markers relevant to male sex determination in mulberry (*Morus alba* L.). Euphytica 215(11):187

Awasthi AK, Nagaraja GM, Naik GV, Kanginakudru S, Thangavelu K, Nagaraju J (2004) Genetic diversity in mulberry (Genus *Morus*) as revealed by RAPD and ISSR marker assays. BMC Genet 5(1):100–104

Bae SH, Suh HJ (2007) Antioxidant activities of five different mulberry cultivars in Korea. Food Sci Technol 40(6):955–962

Baligar VC, Bennett OL (1986) Outlook on fertilizer use efficiency in the tropics. Fert Re 10(1):83–96

Baligar VC, Fageria NK, He ZL (2001) Nutrient use efficiency in plants. Commun Soil Sci Plant Anal 32(7–8):921–950

Banerjee R, Roychoudhury S, Sau H, Das BK, Saha AK, Saratchandra B, Bajpai AK (2011) Phenotypic divergence in mulberry (*Morus* spp.) germplasm collections. J Crop Improv 25(5):459–471

Banerjee R, Chattopadhyay S, Saha AK (2016) Genetic diversity and relationship of mulberry genotypes revealed by RAPD and ISSR markers. J Crop Improv 30(4):478–492. https://doi.org/10.1080/15427528.2016.1177803

Baranwal VK, Khurana P (2016) Genome-wide analysis, expression dynamics and varietal comparison of NAC gene family at various developmental stages in *Morus notabilis*. Mol Genet Genom 291(3):1305–1317. https://doi.org/10.1007/s00438-016-1186-z

Baranwal VK, Negi N, Khurana P (2016) Genome-wide identification and structural, functional and evolutionary analysis of WRKY components of mulberry. Sci Rep 6:30794. https://doi.org/10.1038/srep30794

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297

Baruah B, Gogoi M (2013) Economics of silk industry: an overview. Intl Res J Agri Econ Stat 4(2):196–199

Basten CJ, Weir BS, Zeng ZB (1998) QTL cartographer a reference manual and tutorial for QTL mapping. Department of Statistics North Carolina State University. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

Benjamin JG, Porter LK, Duke HR, Ahuja LR (1997) Corn growth and nitrogen uptake with furrow irrigation and fertilizer bands. Agron J 89(4):609–612

Bhattacharya E, Ranade SA (2001) Molecular distinction amongst varieties of mulberry using RAPD and DAMD profiles. BMC Plant Biol 1(1):3

Bindroo BB, Tiku AK, Pandit RK (1990) Variation of some metrical traits in mulberry varieties. Indian J Forester 116(4):320–324

Bonnet E, Van de Peer Y, Rouzé P (2006) The small RNA world of plants. New Phytol 171(3):451–468

Briggs LJ, Shantz HL (1913) The water requirement of plants. Bureau of plant industry bulletin. US Department of Agriculture, Washington, DC, pp 282–285

Broadfoot WM, Williston HL (1973) Flooding effects on southern forests. J Forestry 71(9):584–587

Burch Smith TM, Anderson JC, Martin GB, Dinesh Kumar SP (2004) Applications and advantages of virus induced gene silencing for gene function studies in plants. Plant J 39(5):734–746

Chaitanya KV, Sundar D, Reddy AR (2001) Mulberry leaf metabolism under high temperature stress. Biol Planta 44(3):379–384

Chaitanya KV, Jutur PP, Sundar D, Reddy AR (2003) Water stress effects on photosynthesis in different mulberry cultivars. Plant Growth Regul 40(1):75–80

Chakraborti SP, Vijayan K, Roy BN, Quadri SMH (1998) *In vitro* induction in tetraploidy in mulberry (*Morus alba* L). Plant Cell Rep 17(10):794–803

Chakraborti SP, Das C, Vijayan K, Misra AK, Raghunath MK, Narayan P, Roy BN (1999) Field evaluation of somaclonal variant (SV1) developed from *Morus alba* L. var. S1. Indian J Sericul 38:69–71

Chang LW, Juang LJ, Wang BS, Wang MY, Tai HM, Hung WJ, Chen YJ, Huang MH (2011) Antioxidant and antityrosinase activity of mulberry (*Morus alba* L.) twigs and root bark. Food Chem Toxicol 49(4):785–790

Chang LY, Li KT, Yang WJ, Chang JC, Chang MW (2014) Phenotypic classification of mulberry (*Morus*) species in Taiwan using numerical taxonomic analysis through the characterization of vegetative traits and chilling requirements. Sci Hort 176:208–217

Chatterjee SN, Nagaraja GM, Srivastava PP, Naik GY (2004) Morphological and molecular variation of *Morus laevigata* in India. Genetica 121(2):133–143

Checker VG, Khurana P (2013) Molecular and functional characterization of mulberry EST encoding remorin (MiREM) involved in abiotic stress. Plant Cell Rep 32(11):1729–1741

Checker VG, Saeed B, Khurana P (2012) Analysis of expressed sequence tags from mulberry (*Morus indica*) roots and implications for comparative transcriptomics and marker identification. Tree Genet Genomes 8(6):1437–1450

Cheema J, Noel Ellis TH, Dicks J (2010) THREaD Mapper Studio: a novel, visual web server for the estimation of genetic linkage maps. Nucleic Acid Res 38 (suppl_2):W188–W193

Cheeseman JM (1988) Mechanisms of salinity tolerance in plants. Plant Physiol 87(3):547–550

Chen J, Chen JZ, Tan MZ, Gong ZT (2002) Soil degradation: a global problem endangering sustainable development. J Geograpgh Sci 12(2):243–252

Chen PN, Chu SC, Chiou HL, Kuo WH, Chiang CL, Hsieh YS (2006) Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer Lett 235(2):248–529

Chen PJ, Senthilkumar R, Jane WN, He Y, Tian Z, Yeh KW (2014) Transplastomic *Nicotiana benthamiana* plants expressing multiple defense genes encoding protease inhibitors and chitinase display broad-spectrum resistance against insects, pathogens and abiotic stresses. Plant Biotechnol J 12(4):503–515

Chen C, Zhou W, Huang Y, Wang ZZ (2016) The complete chloroplast genome sequence of the mulberry *Morus notabilis* (Moreae). Mitochon DNA Part A 27(4):2856–2857

Chen L, Hou Y, Hu W, Qiu X, Lu H, Wei J, Yu S, He N, Zhang H, Shen (2018) The molecular chaperon AKR2A increases the mulberry chilling-tolerant capacity by maintaining SOD activity and unsaturated fatty acids composition. Sci Rep 8(1):1–11

Chikkaswamy BK, Prasad MP (2012) Evaluation of genetic diversity and relationships in mulberry varieties using RAPD and ISSR molecular markers. Intl J Mol Biol 3:2–70

Chikkaswamy BK, Paramanik RC, Debnath A, Sadana MS (2012) Evaluation of genetic diversity in mulberry varieties using molecular markers. Nat Sci 10:45–60

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

Choudhary R, Chaudhury R, Malik SK, Kumar S, Pal D (2013) Genetic stability of mulberry germplasm after cryopreservation by two-step freezing technique. Afr J Biotechnol 12(41):5983–5993

Chu Y, K. Luos C, Wang Y, Yang J (2001) The variety DL-number 1. In: Mulberry for animal feeding in China, proceeding of a workshop, Hangzhou, China, May 14–17, 2001, pp 63–67

Collins FS, Guyer MS, Chakravarti A (1997) Variations on a theme: cataloging human DNA sequence variation. Science 278(5343):1580–1581

Colmer TD, Voesenek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. Funct Plant Biol 36(8):665–681

Das M, Chauhan H, Chhibbar A, Haq QMR, Khurana P (2011) High efficiency transformation and selective tolerance against biotic and abiotic stress in mulberry, *Morus indica* cv. K-2, by constitutive and inducible expression of tobacco Osmotin. Transgen Res 20(2):231–246

De Givry S, Bouchez M, Chabrier P, Milan D, Schiex T (2005) CARHTA GENE: multi-population integrated genetic and radiation hybrid mapping. Bioinformatics 21(8):1703–1704

Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55(4):997–1004

Dorcus D, Vivekanandan M (1997) Exploitation of mulberry genotypes for drought resistance potential. J Sericult Sci Jpn 66(2):71–80

Duan K, Luo YH, Luo D, Xu ZH, Xue HW (2005) New insights into the complex and coordinated transcriptional regulation networks underlying rice seed development through cDNA chip-based analysis. Plant Mol Biol 57(6):785–804

Echt C, Knapp S, Liu BH (1992) Genome mapping with non-inbred crosses using Gmendel 2.0. Maize Genet Coop Newsl 66:27–29

Ercisli S, Orhan E (2007) Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. Food Chem 103(4):1380–1384

Espinosa MJ, Garcia MJP (2009) Tools to improve nutrient use efficiency in corn in tropical Latin America. In: Espinosa J, García F (eds) Proceedings of the symposium "nutrient use efficiency" presented by the International Plant Nutrition Institute (IPNI) at the XVIII Latin American congress of soil science, San José, Costa Rica, November 16–20, 2009, pp 47–54

Fageria NK (1992) Maximizing crop yields. Marcel Dekker, New York

Fang LJ, Qin RL, Liu Z, Liu CR, Gai YP, Ji XL (2019) Expression and functional analysis of a PR-1 Gene, MuPR1, involved in disease resistance response in mulberry (*Morus multicaulis*). J Plant Interact 14(1):376–385

Farnir F, Coppieters W, Arranz JJ, Berzi P, Cambisano N, Grisart B, Karim L, Marcq F, Moreau L, Mni M, Nezer C (2000) Extensive genome-wide linkage disequilibrium in cattle. Genome Res 10(2):220–227

Fernie AR, Schauer N (2008) Metabolomics-assisted breeding: a viable option for crop improvement? Trend Genet 25(1):39–48

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants-where next. Aust J Plant Physiol 22(6):875–884

Frankel OH, Soule ME (1981) Conservation and evolution. Cambridge Univ Press, London

Fukuda T, Sudo M, Matuda M, Hayashi T, Kurose T, Horiuhi MF (1959) Formation of silk protein during the growth of the silkworm larvae, *Bombyx mori* L. In: Proceedings of the 4th Instar congress of bio-chemistry, vol 12, pp 90–112

Garcia-Gómez B, González-Alvarez H, Martínez-Mora C, Cenis LJ, Pérez-Hernández CDM, Martínez-Zubiaur Y, Martínez-Gómez P (2019) The molecular characterization of an extended mulberry germplasm by SSR markers. Genetika 51(2):389–403

Ghosh MK, Misra AK, Shivnath DC, Sengupta T, Ghosh PK, Singh MK, Bindroo BB (2012) Morpho-physiological evaluation of some mulberry genotypes under high moisture stress condition. J Crop Weed 8(1):167–170

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48(12):909–930

Gnanesh BN, Tejaswi A, Arunakumar GS, Supriya M, Manojkumar HB, Tewary P (2021) Molecular phylogeny, identification and pathogenicity of *Rhizopus oryzae* associated with root rot of mulberry in India. J Appl Microbiol 131(1):360–374

Golombek SD, Lüdders P (1993) Effect of short-term salinity on leaf gas exchange of the fig (*Ficus carica* L.). Plant Soil 148(1):21–27

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of Eucalyptus grandis and Eucalyptus urophylla using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137(4):121–1137

Gucci R, Lombardini L, Tattini M (1997) Analysis of leaf water relations of two olive (Olea europaea) cultivars differing in tolerance to salinity. Tree Physiol 17(7):490–490

Guha A, Sengupta D, Rasineni G, Reddy AR (2009) An integrated diagnostic approach to understand drought tolerance in mulberry (*Morus indica* L.). Flora 205(2):144–151

Guha A, Rasineni G, Reddy AR (2010) Drought tolerance in mulberry (*Morus* spp.): a physiological approach with insights into growth dynamics and leaf yield production. Exp Agri 46(4):471–488

Gulyani V, Khurana P (2011) Identification and expression profiling of drought-regulated genes in mulberry (*Morus* sp.) by suppression subtractive hybridization of susceptible and tolerant cultivars. Tree Genet Genomes 7(4):725–738

Halushka MK, Fan JB, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, Chakravarti A (1999) Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. Nat Genet 22(3):239–247

Hartl D, Clark AG (1989) Principles of population genetics, 2nd edn. Sinauer Associates, Sunderland

Hassimotto NMA, Genovese MI, Lajolo FM (2007) Identification and characterization of anthocyanins from wild mulberry (*Morus nigra* L.) growing in Brazil. Food Sci Technol Intl 13(1):17–25

Hautala EL, Wulff A, Oksanen J (1992) Effects of deicing salt on visible symptoms, element concentrations and membrane damage in first-year needles of roadside Scots pine (*Pinus sylvestris*). Ann Bot Fenn 29:179–185

He N, Zhang C, Qi X, Zhao S, Tao Y, Yang G, Lee TH, Wang X, Cai Q, Li D, Lu M, Liao S, Luo G, He R, Tan X, Xu Y, Li T, Zhao A, Jia L, Fu Q, Zeng Q, Gao C, Ma B, Liang J, Wang X, Shang J, Song P, Wu H, Fan L, Wang Q, Shuai Q, Zhu J, Wei C, Zhu- Salzman K, Jin D, Wang J, Liu T, Yu M, Tang C, Wang Z, Dai F, Chen J, Liu Y, Zhao S, Lin T, Zhang S, Wang J, Wang J, Yang H, Yang G, Wang J, Paterson AH, Xia Q, Ji D, Xiang Z (2013) Draft genome sequence of the mulberry tree *Morus notabilis*. Nat Commun 4(1):1–9. https://doi.org/10.1038/ncomms3445

Hill WG (1974) Estimation of linkage disequilibrium in randomly mating populations. Heredity 2:229–239

Hirano H (1982) Varietal differences of leaf protein profiles in mulberry. Phytochemistry 21(7):1513–1518

Hirano H, Naganuma K (1979) Inheritance of peroxidase isozymes in mulberry (*Morus* spp.). Euphytica 28(1):73–79

Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the post genome era: past, present and future. Plant J 61(6):1041–1052

Hong JH, Ahn JM, Park SW, Rhee SJ (2004) The effects of mulberry fruit on the antioxidative defense systems and oxidative stress in the erythrocytes of streptozotocin-induced diabetic rats. Nutri Sci 7(3):127–132

Hossain M, Rahama SM, Jorder OI (1991) Isolation of sodium chloride resistant genotypes in mulberry cultivars. Bull Sericult Res 2:67–73

Hou H, Atlihan N, Lu ZX (2014) New biotechnology enhances the application of cisgenesis in plant breeding. Front Plant Sci 5:389

Huang XQ, Börner A, Röder MS, Ganal MW (2002) Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor Appl Genet 105(5):699–707

Huang RZ, Yan X-P, Li J, Zhang XW (2009) AFLP finger print analysis for 10 mulberry cultivars in Hunan province. Sci Sericult 35:837–841

Huang G, Zeng Y, Wei L, Yongquan Y, Dai J, Liu G, Gui A (2020) Comparative transcriptome analysis of mulberry reveals anthocyanin biosynthesis mechanisms in black (*Morus atropurpurea* Roxb.) and white (*Morus alba* L.) fruit genotypes. BMC Plant Biol 20:279. https://doi.org/10.1186/s12870-020-02486-1

Huschke RE (1959) Glossary of meteorology. American Meteorological Society, Boston

Imran M, Khan H, Shah M, Khan R, Khan F (2010) Chemical composition and antioxidant activity of certain *Morus* species. J Zhejiang Univ Sci B 11(12):973–980

Ipek M, Pirlak L, Kafkas S (2012) Molecular characterization of mulberry (*Morus* spp.) genotypes via RAPD and ISSR. J Sci Food Agri 92(8):1633–1637

Israel DW, Rufty Junior TW (1988) Influence of phosphorus nutrition on phosphorus and nitrogen utilization efficiencies and associated physiological response in soybean. Crop Sci 28(6):954–960

Jana R (2010) Potential of chloroplast genome in plant breeding. Czech J Genet Plant Breed 46(3):103–113

Jennings HS (1917) The numerical results of diverse systems of breeding, with respect to two pairs of characters, linked or independent, with special relation to the effects of linkage. Genetics 2(2):97–154

Jhansi Lakshmi K, Anandarao A, Borpuzari MM (2014) Potential mulberry genetic resources for drought tolerance. Paper presented in 23rd International congress on sericulture and silk industry held on 24–27th November, 2014, Bangalore, India

Jiang D, Sui Y, Lang X (2016) Timing and associated climate change of a 2 °C global warming. Intl J Climatol 36(14):4512–4522

Jiang SM, Huang RZ, Jiang YB, Li ZB (2020) Comparative transcriptome analysis profiles of two mulberry varieties under cadmium stress. Russ J Plant Physiol 67(6):1126–1134. https://doi.org/10.1134/S1021443720060096

Jiao F, Luo R, Dai X, Liu H, Yu G, Han S, Lu X, Su C, Chen Q, Song Q, Meng C, Li F, Sun H, Zhang R, Hui T, Qian Y, Zhao A, Jiang Y (2020) Chromosome level reference genome and population genomic analysis provide insight into the evolution and improvement of domesticated mulberry (*Morus alba* L). Mol Plant 13(7):1001–1012

Jones Q (1983) Germplasm needs of oilseed crops. Econ Bot 37:418–422

Jourjon MF, Jasson S, Marcel J, Ngom B, Mangin B (2005) MCQTL: multi-allelic QTL mapping in multi-cross design. Bioinformatics 21(1):128–130

Jung C, Seo JS, Han SW, Koo YJ, Kim CH, Song SI, Nahm BH, Choi YD, Cheong JJ (2007) Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis. Plant Physiol 146(2):623–635

Jungk A (2001) Root hairs and the acquisition of plant nutrients from soil. J Plant Nutri Soil Sci 164(2):121–129

Kafkas S, Ozgen M, Dogan Y, Özcan B, Ercişli S, Serce S (2008) Molecular characterization of mulberry accessions in Turkey by AFLP markers. J Amer Soc Hort Sci 133(4):593–597

Kang TH, Hur JY, Kim HB, Ryu JH, Kim SY (2006) Neuroprotective effects of the cyanidin-3-O-β-d-glucopyranoside isolated from mulberry fruit against cerebral ischemia. Neurosci Lett 3:122–126

Kar P, Srivastava PP, Awasthi AK, Urs SR (2008) Genetic variability and association of ISSR markers with some biochemical traits in mulberry (*Morus* spp.) genetic resources available in India. Tree Genet Genomes 4(1):75–83

Katsumata F (1979) Chromosomes of *Morus nigra* L. from Java and hybridization affinity between this species and some mulberry species in Japan. J Sericult Sci Jpn 48(5):418–422

Kefu Z, Munns R, King RW (1991) Abscisic acid levels in NaCl-treated barley, cotton, and saltbush. Aust J Plant Physiol 18(1):17–24

Khin MM, Nair AS, Babu VJ, Murugan R, Ramakrishna S (2012) A review on nanomaterials for environmental remediation. Energy Environ Sci 5(8):8075–8109

Kim S, Kim D, Cho SW, Kim J, Kim JS (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. Genome Res 24(6):1012–1019

Kong W, Yang J (2016) The complete chloroplast genome sequence of *Morus mongolica* and a comparative analysis within the *Fabidae* clade. Curr Genet 62(1):165–172

Kong WQ, Yang JH (2017) The complete chloroplast genome sequence of *Morus cathayana* and *Morus multicaulis*, and comparative analysis within genus *Morus* L. Peer J 5:e3037. https://doi.org/10.7717/peerj.3037

Kramer PJ (1983) Water relations of plants. New York Academic Press, New York

Krishnan RR, Naik VG, Ramesh SR, Qadri SMH (2013) Microsatellite marker analysis reveals the events of the introduction and spread of cultivated mulberry in the Indian subcontinent. Plant Genet Resour 12(1):129–139

Krishnan RR, Dandin SB, Naik VG (2014a) Group wise sampling: a strategy to sample core entries from RAPD marker data with application to mulberry. Trees 28(3):723–731

Krishnan RR, Sumathy R, Bindroo BB, Naik VG (2014b) MulSatDB: a first online database for mulberry microsatellites. Trees 28(6):1793–1799

Kumar GS, Lakshmi A, Madhusudan KV, Ramanjulu S, Sudhakar C (1999) Photosynthesis parameters in two cultivars of mulberry differing in salt tolerance. Photosynthetica 36(4):611–616

Kumar GSA, Reddy M, Sudhakar C (2003) NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Sci 165(6):1245–1251

Lakshmanan S (2007) Growth trends in mulberry silk production in India—an economic analysis. Finan Agri 39(4):28–30

Lakshmi A, Ramanjulu S, Veeranjaneylu K, Sudhakar C (1996) Effect of NaCl on photosynthesis parameters in two cultivars of mulberry. Photosynthetica 32:285–289

Lal S, Gulyani V, Khurana P (2008) Overexpression of HVA1 gene from barley generates tolerance to salinity and water stress in transgenic mulberry (*Morus indica*). Transgen Res 17(4):651–663

Lal S, Ravi V, Khurana JP, Khurana P (2009) Repertoire of leaf expressed sequence tags (ESTs) and partial characterization of stress-related and membrane transporter genes from mulberry (*Morus indica* L.). Tree Genet Genomes 5(2):359–374

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121(1):185–199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1(2):174–181

Lavanya C, Ashoka J, Sreenivasa AG, Sushila N, Beladhadi BV (2017) Effect of elevated carbon dioxide and temperature on growth, yield and quality parameters of mulberry. Entomol Ornithol Herpetol 6(2):1–3. https://doi.org/10.4172/2161-0983.1000196

Le Houerou, HN (1980) Browse in Africa: the current state of knowledge. ILCA, Addis Ababa

Leopold AC, Willing RP (1984) Evidence for toxicity effects of salt on membranes. In: Staples RC, Toenniessen GH (eds) Salinity tolerance in plants. Strategies for crop improvement. Wiley Interscience, New York, pp 67–76

Levitt J (1972) Responses of plants to environmental stresses, vol xiv. Academic Press, New York, p 698

Lewontin RC (1964) The interaction of selection and linkage. I. General considerations; heterotic models. Genetics 49(1):49–67

Li JF, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J (2013) Multiplex and homologous recombination–mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. Nat Biotechnol 31(8):688–691

Li T, Qi X, Zeng Q, Xiang Z, He N (2014) MorusDB: a resource for mulberry genomics and genome biology. Database, p bau054

Li QL, Guo JZ, Yan N, Li CC (2016) Complete chloroplast genome sequence of cultivated *Morus* L. species. Genet Mol Re 15:gmr15048906

Li R, Chen D, Wang T, Wan Y, Li R, Fang R, Wang Y, Hu F, Zhou H, Li L, Zhao W (2017) High throughput deep degradome sequencing reveals microRNAs and their targets in response to drought stress in mulberry (*Morus alba*). PLoS ONE 12(2):e0172883

Li R, Liu L, Dominic K, Wang T, Fan T, Hu F, Wang Y, Zhang L, Li L, Zhao W (2018a) Mulberry (*Morus alba*) MmSK gene enhances tolerance to drought stress in transgenic mulberry. Plant Physiol Biochem 132:603–611

Li R, Fan T, Wang T, Dominic K, Hu F, Liu L, Zhang L, Fang R, Pan G, Li L, Zhao W (2018b) Characterization and functional analysis of miR166f in drought stress tolerance in mulberry (*Morus multicaulis*). Mol Breed 38(11):132

Li H, Dong Li D, Yang Z, Zeng Q, Luo Y, He N (2020a) Flavones produced by mulberry flavone synthase type I constitute a defense line against the ultraviolet-B stress. Plants 9(2):215. https://doi.org/10.3390/plants9020215

Li D, Chen G, Ma B, Zhong C, He N (2020b) Metabolic profiling and transcriptome analysis of mulberry leaves provide insights into flavonoid biosynthesis. J Agri Food Chem 68(5):1494–1504

Li D, Ma B, Xu X, Chen G, Li T, He N (2020c) MMHub, a database for the mulberry metabolome. Database (Oxford) 2020b:baaa011. https://doi.org/10.1093/database/baaa011

Li R, Hu F, Li B, Zhang Y, Chen M, Fan T, Wang T (2020d) Whole genome bisulfite sequencing methylome analysis of mulberry (*Morus alba*) reveals epigenome modifications in response to drought stress. Sci Rep 10(1):1–17

Lin Z, Guo Z, Bai C, Yong H, Jia S, Li L, Sheng Q (2011) Analysis of genetic diversity and construction of core collection of local mulberry varieties from Shanxi Province based on ISSR marker. Afr J Biotechnol 10(40):7756–7765

Lippman Z, Martienssen R (2004) The role of RNA interference in heterochromatic silencing. Nature 431(7006):364–370

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Amer J Hum Genet 44(3):397

Liu YG, Shirano Y, Fukaki H, Yanai Y, Tasaka M, Tabata S, Shibata D (1999) Complementation of plant mutants with large genomic DNA fragments by a transformation-competent artificial chromosome vector accelerates positional cloning. Proc Natl Acad Sci USA 96:6535–6540

Liu XQ, Liu CY, Guo Q, Zhang M, Cao BN, Xian ZH, Zhao AC (2015) Mulberry transcription factor mndreb4a confers tolerance to multiple abiotic stresses in transgenic tobacco. PLoS ONE 10(12):e0145619

Liu CY, Liu XQ, Long DP, Cao BN, Xiang ZH, Zhao AC (2017) De novo assembly of mulberry (*Morus alba* L.) transcriptome and identification of candidate unigenes related to salt stress responses. Russ J Plant Physiol 64(5):738–748

Liu Y, Ji D, Turgeon R, Chen J, Lin T, Huang J, Luo J, Zhu Y, Zhang C, Lv Z (2019) Physiological and Proteomic responses of mulberry trees (*Morus alba.* L.) to combined salt and drought stress. Intl J Mol Sci 20(10):2486

Lopes AS, Guilherme LRG (2000) Uso eficiente de fertilizantes e corretivos agrícolas: aspectos agronômicos, 3 edn. rev. ANDA, São Paulo, 72 pp (*Boletim Técnico*, 4)

Lu X, Sun B, Shen F, Pan G, Wang J, Lou C (2008) Cloning of low-temperature induced gene from Morus mongolica CK Schn and its transformation into *Petunia hybrida* Vilm. Afr J Biotechnol 7(5)

Lu N, Luo Z, Ke Y, Dai L, Duan H, Hou R, Cui B, Dou S, Zhang Y, Sun Y, Li Y (2017) Growth, physiological, biochemical and ionic responses of *Morus alba* L. seedlings to various salinity levels. Forests 8(12):488

Machado AT (1997) Perspectiva do melhoramento genético em milho (*Zea mays* L.) visando eficiência na utilização do nitrogênio. Tese (Doutorado em Genética e Melhoramento de Plantas) - Curso de Pós-Graduação em Genética e Melhoramento, Universidade Federal do Rio de Janeiro, Rio de Janeiro

Machii M (1990) Leaf disc transformation of mulberry plant (*Morus alba* L.) by *Agrobacterium* Ti plasmid. J Sericult Sci Jpn 59(2):105–110

Mackay L, Powell W (2007) Methods for linkage disequilibrium mapping in crops. Trends Plant Sci 12(2):57–63

Madhuri T (2015) Discovery of QTLs for drought tolerance traits using a biparental mapping population of mulberry segregating for root and water use efficiency (WUE). PhD thesis, Uni of Agri Sci, GKVK, Bengaluru. https://krishikosh.egranth.ac.in/handle/1/5810029246

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27(2 Part 1):209–220

Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu JK (2013) Application of the CRISPR–Cas system for efficient genome engineering in plants. Mol Plant 6(6):2008–2011

Margarido GRA, Souza AP, Garcia AAF (2007) OneMap: software for genetic mapping in outcrossing species. Hereditas 144(3):78–79

Matsui A, Ishida J, Morosawa T, Okamoto M, Kim JM, Kurihara Y, Kawashima M, Tanaka M, To TK, Nakaminami K, Kaminuma E (2010) Arabidopsis tiling array analysis to identify the stress-responsive genes. In: Sunkar R (ed) Plant stress tolerance. Humana Press, pp 141–155

Machii H, Koyama A, Yamanouchi H (1999) A list of genetic mulberry resources maintained at National Institute of Sericultural and Entomological Science. Miscellaneous Publ Nat Sericultural Entomol Sci 26:1–77

Mc Murphy LM, Rayburn AL (1992) Chromosomal and cell size analysis of cold tolerant maize. Theor Appl Genet 84(7–8):798–802
McKersie D, Leshem Y (1994) Stress and stress coping in cultivated plants. Kluwer Academic Publishers, London
Mishra S (2014) Genetic analysis of traits controlling water use efficiency and rooting in mulberry (*Morus* spp.) by molecular markers. PhD thesis, University of Mysore, Mysuru, India
Mishra S, Naik VG, Sukumar M, Pinto MV, Sheshashayee MS, Dandin SB (2013) Genetic analysis of parental genotypes for mapping of water use efficiency and root traits in mulberry. Indian J Genet 73(4):405–410
Misra AK, Ghosh MK, Bindroo BB (2012) Water deficit condition and its impact on mulberry plant (*Morus* spp.) in relation to its growth and physiological parameters. J Crop Weed 8(2):143–145
Moeller DA, Schaal BA (1999) Genetic relationships among Native American maize accessions of the Great Plains assessed by RAPDs. Theor Appl Genet 99(6):1061–1067
Mohlke KL, Lange EM, Valle TT, Ghosh S, Magnuson VL, Silander K, Watanabe RM, Chines PS, Bergman RN, Tuomilehto J, Collins FS (2001) Linkage disequilibrium between microsatellite markers extends beyond 1 cM on chromosome 20 in Finns. Genome Res 11(7):1221–1226
Muhonja L, Yamanouchi H, Yang C, Kuwazaki S, Yokoi K, Kameda T, Sezutsu H, Jouraku A (2020) Genome-wide SNP marker discovery and phylogenetic analysis of mulberry varieties using double-digest restriction site-associated DNA sequencing. Gene 726:144162
Mustroph A (2018) Improving flooding tolerance of crop plants. Agronomy 8(9):160
Naik VG, Subbulakshmi N, Pinto MV, Mishra S, Guruprasad Qadri SMH (2013) Assessment of genetic diversity among mulberry collections from South India using phenotypic and RAPD markers. Indian J Sericult 52(1):34–43
Naik VG, Thumilan B, Sarkar A, Dandin SB, Pinto MV, Sivaprasad V (2014) Development of genetic linkage map of mulberry using molecular markers and identification of QTLs linked to yield and yield contributing traits. Sericologia 54(4):221–229
Naik VG, Dandin SB, Tikader A, Pinto MV (2015) Molecular diversity of wild mulberry (*Morus* spp.) of Indian subcontinent. Indian J Biotechnol 14(3):334–343
Namkoong G, Kang HC, Brouard JS (1988) Tree breeding: principles and strategies. Springer, Berlin Heidelberg New York
Nanavaty M (1990) Silk production processing and marketing. Wiley Eastern Limited, New Delhi
Nanjo T, Kobayashi M, Yoshida Y, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (1999) Antisense suppression of proline degradation improves tolerance to freezing and Salinity in *Arabidopsis thaliana.* FEBS Lett 461(3):205–210
Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76(10):5269–5273
Nelson JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3(3):239–245
Nepal MP, Ferguson CJ (2012) Phylogenetics of Morus (Moraceae) inferred from ITS and trnL-trnF sequence data. Syst Bot 37(2):442–450
Ni G, Zhang QJ, Wang HY, Chen RY, Zheng FZ, Yu DQ (2010) Chemical constituents of the stem bark of *Morus cathayana.* J Asian Nat Prod Res 12(6):505–515
Nozue M, Cai W, Li L, Xu W, Shioiri H, Kojima M, Saito H (2000) Development of a reliable method for Agrobacterium tumefaciens-mediated transformation of mulberry callus. J Sericult Sci Jpn 69(6):345–352
Ohyama K (1966) Effect of soil moisture on growth of mulberry tree. Bull Sericult Exp Sci Jpn 20:333–359
Oka S, Tewary PK (2000) Induction of hairy roots from hypocotyls of mulberry (*Morus indica* L.) by Japanese wild strains of Agrobacterium rhizogenes. J Sericult Sci Jpn 69(1):13–19
Orhan E, Ercisli S, Yildirim N, Agar G (2007) Genetic variations among mulberry genotypes (*Morus alba*) as revealed by random amplified polymorphic DNA (RAPD) markers. Plant Syst Evol 265(3–4):251–258

Orhan E, Akin M, Eyduran SP, Ercisli S (2020) Molecular characterization of mulberry genotypes and species in Turkey. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48(2):549–557. https://doi.org/10.15835/nbha48211928

Ozrenk K, Sensoy RIG, Erdinc C, Guleryuz M, Aykanat A (2010) Molecular characterization of mulberry germplasm from Eastern Anatolia. Afr J Biotechnol 9(1):1–6

Palaisa K, Morgante M, Tingey S, Rafalski AJ (2004) Long-range patterns of diversity and linkage disequilibrium surrounding the maize Y1 gene are indicative of an asymmetric selective sweep. Proc Natl Acad Sci USA 101:9885–9890

Pan YL (2000) Progress and prospect of germplasm resources and breeding of mulberry. Acta Sericol Sin 26(Suppl):1–8

Pan YL (2003) Popularization of good mulberry varieties and sericultural development. Acta Sericol Sin 1:1–6

Pavan U (2010) Mulberry improvements via plastid transformation and tissue culture engineering. Plant Signal Behav 5(7):785–787

Pennisi E (2013) The CRISPR craze. Science 341:833–836

Peris NW, Gacheri KM, Theophillus MM, Lucas N (2014) Morphological characterization of mulberry (*Morus* spp.) accessions grown in Kenya. Sustain Agri Res 3(526-2016-37790):10–17

Piao H, Li S, Wang S (2016) Nutrient uptake by mulberry and Chinese prickly ash associated with arbuscular mycorrhizal fungi. Acta Geochim 35(2):120–129

Piatek A, Ali Z, Baazim H, Li L, Abulfaraj A, Al Shareef S, Aouida M, Mahfouz MM (2015) RNA guided transcriptional regulation in planta via synthetic dCas9 based transcription factors. Plant Biotechnol J 13(4):578–589

Pierce JC, Sauer B, Sternberg N (1992) A positive selection vector for cloning high molecular weight DNA by the bacteriophage P1 system: improved cloning efficacy. Proc Natl Acad Sci USA 89(6):2056–2060

Pinto MV, HS P, MS R, Naik VG (2018a) Association mapping of quantitative resistance to charcoal root rot in mulberry germplasm. PLoS ONE 13(7):e0200099

Pinto MV, Poornima HS, Sivaprasad V, Naik VG (2018b) A new set of mulberry-specific SSR markers for application in cultivar identification and DUS testing. J Genet 97(1):31–37

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multi locus genotype data. Genetics 155(2):945–959

Pryluckyi OV (1969) Study of metabolism in mulberry varieties of different winter hardiness. Sovkivn Mizrid Nauk Zvrm 5:59–64

Rahman MS, Doss SG, Vijayan K, Setua M, Roy BN (1999) Performance of improved varieties of mulberry under rainfed cultivation in West Bengal. Indian J Agri Sci 69(10):752–754

Ramanjulu S, Sudhakar C (2000) Proline metabolism during dehydration in two mulberry genotypes with contrasting drought tolerance. J Plant Physiol 157(1):81–85

Ramanjulu S, Veeranjulu K, Sudhakar C (1993) Sodium, potassium and nitrogen status of some mulberry (*Morus alba* L.) cultivars under NaCl salinity. Indian J Plant Physiol Biochem 19:103–106

Ramanjulu S, Veeranjaneyulu K, Sudhakar C (1994) Short term shifts in nitrogen metabolism in mulberry (*Morus alba*) under salt shock. Phytochemistry 37(4):991–995

Ramanjulu S, Sreenivasalu N, Giridara KS, Sudhakar C (1998) Photosynthetic characteristics in mulberry during water stress and rewatering. Photosynthetica 35(2):259–263

Rao A, Vijayan K, Krubakaran M, Borpujari MM, Kamble CK (2011) Genetic diversity in mulberry (Morus L.) revealed by isozyme markers. J Hort Sci Biotechnol 86(3):291–297

Rathore MS, Srinivasulu Y (2018) Management of soil fertility for sustaining quality mulberry leaf production in North India. Intl J Sci Res Biol Sci 5(5):58–62

Ravi V, Khurana JP, Tyagi AK, Khurana P (2006) The chloroplast genome of mulberry: complete nucleotide sequence, gene organization and comparative analysis. Tree Genet Genomes 3(1):49–59

Ravi V, Tyagi AK, Khurana JP, Khurana P (2007) Rosales sister to Fabales: towards resolving the rosid puzzle. Mol Phylogenet Evol 44(1):488–493

Reynolds MP, Ortiz R (2010) Adapting crops to climate change: a summary. In: Reynolds MP (ed) Climate change and crop production. CAB International, Wallingford, Oxon, OK, pp 1–8
Rick CW (1984) Plant Germplasm Resources. In: Evens DA, Sharp WR, Ammirato PV, Yamada Y (eds) Handbook of cell culture. MacMillan, New York, pp 9–37
Rieder MJ, Taylor SL, Tobe VO, Nickerson DA (1998) Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome. Nucleic Acid Res 26(4):967–973
Rodríguez C, Arias R, Quiñones J (1994) Efecto de la frecuencia de poda y el nivel de fertilización nitrogenada, sobre el rendimiento y calidad de la biomasa de morera (*Morus* spp.) en el trópico seco de Guatemala. En: Benavides JE (ed) Arboles y arbustos forrajeros en América Central. CATIE, Turrilaba, Costa Rica, pp 515–529
Rohela GK, Jogam P, Shabnam AA, Shukla P, Abbagani S, Mrinal KG (2018) *In vitro* regeneration and assessment of genetic fidelity of acclimated plantlets tby using ISSR markers in PPR-1 (*Morus* sp.): an economically important plant. Sci Hort 241:313–321 e00417
Rohela GK, Jogam P, Mir MY, Shabnam AA, Shukla P, Abbagani S, Azra NK (2020) Indirect regeneration and gentic fidelity analysis of acclimated plantlets through SCoT and ISSR markers in *Morus alba* L. cv. Chinese White. Biotechnol Rep 25:1–12 e00417
Rukmangada MS, Sumathy R, Kruthika HS, Naik VG (2020) Mulberry (*Morus* spp.) growth analysis by morpho-physiological and biochemical components for crop productivity enhancement. Sci Hort 259:108819
Saeed B, Khurana P (2016) Transcription activation activity of ERD15 protein from *Morus indica*. Plant Physiol Biochem 111:174–178
Saeed B, Das M, Haq QMR, Khurana P (2015) Over expression of beta carotene hydroxylase-1 (bch1) in mulberry, *Morus indica* cv. K2, confers tolerance against high-temperature and high-irradiance stress induced damage. Plant Cell Tiss Org Cult 120(3):1003–1015
Saeed B, Baranwal VK, Khurana P (2016) Comparative transcriptomics and comprehensive marker resource development in mulberry. BMC Genomics 17(1):98. https://doi.org/10.1186/s12864-016-2417-8
Saha S, Adhikari S, Dey T, Ghosh P (2016) RAPD and ISSR based evaluation of genetic stability of micropropagated plantlets of *Morus alba* L. variety S-1. Meta Gene 7:7–15
Sahu PK, Yadav BRD, Saratchandra B (1995) Evaluation of yield components in mulberry germplasm varieties. Acta Bot 23:191–197
Sajeevan RS, Nataraja KN (2016) Molecular cloning and characterization of a novel basic helix–loop–helix-144 (bHLH144)-like transcription factor from *Morus alba* (L.). Plant Gene 5:109–117
Sakai A, Larcher W (1987) The freezing process in plants. In: Billings WD, Golley F, Lange OL, Olson JS, Remmert H (eds) Forest survival of plants. Responses and adaptation to freezing stress. Springer, Berlin, pp 21–38
Sanchez MD (2000a) World distribution and utilization of mulberry, potential for animal feeding. In: FAO electronic conference on mulberry for animal production, Rome, Italy, 1. (*Morus*-L). http://www.fao.org/DOCREP/005/X9895E/x9895e02.htm
Sanchez MD (2000b) Mulberry: an exceptional forage available almost worldwide. World Anim Rev 93(1), FAO, Rome
Sanjappa M (1989) Geographical distribution and exploration of the genus *Morus* L. (Moraceae). In: Sengupta K, Dandin SB (eds) Genetic resources of mulberry and utilization. Jwalamukhi Job Press, Bangalore, pp 4–7
Sarkar T, Radhakrishnan T, 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
Sarkar T, Mogili T, Sivaprasad V (2017) Improvement of abiotic stress adaptive traits in mulberry (*Morus* spp.): an update on biotechnological interventions. Biotechnology 7(3):214
Sarkar T, Thankappan R, Mishra GP, Nawade BD (2019) Advances in the development and use of DREB for improved abiotic stress tolerance in transgenic crop plants. Physiol Mol Biol Plant 25:1323–1334

Sasidharan R, Bailey-Serres J, Ashikari M, Atwell BJ, Colmer TD, Fagerstedt K, Fukao T, Geigenberger P, Hebelstrup KH, Hill RD, Holdsworth MJ (2017) Community recommendations on terminology and procedures used in flooding and low oxygen stress research. New Phytol 214:1403–1407

Sastry CR (1984) Mulberry varieties, exploitations and pathology. Sericologia 24(3):333–359

Sastry CR, Venkataramu CV, Khan A, Rao JK (1974) Chemical mutagenesis for productive breeding in mulberry. Paper presented at the seminar organized in commemoration of silver jubilee of Central Silk Board, Bangalore, India, p 20

Schouten HJ, Krens FA, Jacobsen E (2006) Cisgenic plants are similar to traditionally bred plants: International regulations for genetically modified organisms should be altered to exempt cisgenesis. EMBO Rep 7(8):750–753

Seaton G, Haley CS, Knott SA, Kearsey M, Visscher PM (2002) QTL Express: mapping quantitative trait loci in simple and complex pedigrees. Bioinformatics 18(2):339–340

Sekhon BS (2014) Nanotechnology in agri-food production: an overview. Nanotechnol Sci Appl 7:31–53

Serraj R, Sinclair TR (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ 25(2):333–341

Shabnam AA, Fotadar RK, Dhar A, Bhat AH (2012) A Comparative analysis to tropical and temperate genotypes and behaviour of five species of mulberry under temperate conditions of Kashmir. Res J Agri Sci 3(1):089–093

Shabnam AA, Rathore MS, Dhar A, Srinivasulu Y, Chauhan SS, Sharma SP (2016) Mulberry (*Morus spp.*) diversity in Jammu and Kashmir. Indian Hort J 6(1):48–52

Shabnam AA, Chauhan S, Khan G, Shukla P, Saini P, Ghosh M (2018) Mulberry breeding strategies for North and North West India. Intl J Adv Res Sci Eng 7:2124–2133

Shannon MC, Grieve CM (1999) Tolerance of vegetable crops to salinity. Sci Hort 78(1–4):5–38

Shannon MC, Grieve CM, Francois LE (1994) Whole-plant response to salinity. In: Wilkinson RE (ed) Plant-environment interactions. Dekker Marcel, New York, pp 199–244

Sharma AC, Sharma R, Machii H (2000) Assessment of genetic diversity in a Morus germplasm collection using fluorescence-based AFLP markers. Theor Appl Genet 101(7):1049–1055

Sheshshayee MS, Bindumadhava H, Shangar AG, Prasad TG, Udayakumar M (2003) Breeding strategies to exploit WUE for crop improvement. J Plant Biol 30(2):253–268

Shukla P, Rohela GK, Shabnam AA, Sharma SP (2016) Prospect of cold tolerant genes and its utilization in mulberry improvement. Indian Hort J 6:127–129

Shukla P, Reddy RA, Ponnuvel KM, Rohela GK, Shabnam AA, Chauhan SS, Ghosh MK, Mishra RK (2018) Comparative analysis of gene expression profiles among contrasting mulberry varieties under cold stress condition. J Exp Biol Agri Sci 6(6):973–982

Shukla P, Reddy RA, Ponnuvel KM, Rohela GK, Shabnam AA, Ghosh MK, Mishra RK (2019) Selection of suitable reference genes for quantitative real-time PCR gene expression analysis in Mulberry (*Morus alba* L.) under different abiotic stresses. Mol Biol Rep 46(1):1–9

Sinclair TR, Tanner CB, Bennett JM (1984) Water-use efficiency in crop production. Bioscience 34(1):36–40

Singh AK, Singh RA (1999) Effect of salinity of photosynthetic pigments in chickpea (*Cicer arietinuml.*) Leaves. Indian J Plant Physiol 4(1):49–51

Singhal BK, Dhar A, Khan MA, Bindroo BB, Fotedar RK (2009) Potential economic additions by mulberry fruits in sericulture industry. Plant Hort Technol 9(1):47–51

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58

Song W, Wang HJ, Bucheli P, Zhang PF, Wei DZ, Lu YH (2009) Phytochemical profiles of different mulberry (Morus sp.) species from China. J Agri Food Chem 57(19):9133–9140

Srivastava PP, Vijayan K, Awasthi AK, Saratchandra B (2004) Genetic analysis of *Morus alba* through RAPD and ISSR markers. Indian J Biotechnol 3:527–532

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: Join Map. Plant J 3(5):739–744

Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF, Lambert W, Van Der Straeten D (2007) Folate fortification of rice by metabolic engineering. Nat Biotechnol 25(11):1277–1279

Sudhakar P, Chattopadhyay GN, Gangwar SK, Ghosh JK (2000) Effect of foliar application of *Azatobacter, Azospririllum* and *Beijerinkia* on leaf yield and quality of mulberry (*Morus alba*). J Agri Sci 134(2):227–234

Sun H, Zhao W, Liu H, Su C, Qian Y, Jiao F (2020) MaCDSP32 from mulberry enhances resilience post-drought by regulating antioxidant activity and the osmotic content in transgenic tobacco. Front Plant Sci 11:419

Susheelamma BN, Jolly MS, Giridhar K, Sengupta K (1990) Evaluation of germplasm genotypes for drought resistance in mulberry. Sericologia 30(3):327–346

Susheelamma BN, Kumar JS, Mogili T, Sengupta K, Padma MN, Suryanarayana N (1992) Evaluation techniques for screening for drought resistance in mulberry. Sericologia 32(4):609–614

Susheelamma BN, Shekhar KR, Sarkar A, Rao MR, Datta RK (1996) Genotypes and hormonal effects on callus formation and regeneration in mulberry. Euphytica 90(1):25–29

Szabolcs I (1994) Salt affected soils as the ecosystem for halophytes. In: Halophytes as a resource for livestock and for rehabilitation of degraded lands. Springer, Dordrecht, pp 19–24

Tattini M, Gucci R, Coradeschi MA, Ponzio C, Everard JD (1995) Growth, gas exchange and ion content in *Olea europaea* plants during salinity stress and subsequent relief. Physiol Planta 95(2):203–210

Tewary PK, Sharma A, Raghunath MK, Sarkar A (2000) *In vitro* response of promising mulberry (Morus sp.) genotypes for tolerance to salt and osmotic stresses. Plant Growth Regul 30(1):17–21

Thimmanaik S, Kumar SG, Kumari GJ, Suryanarayana N, Sudhakar C (2002) Photosynthesis and the enzymes of photosynthetic carbon reduction cycle in mulberry during water stress and recovery. Photosynthetica 2:233–236

Thumilan M, Kadam NN, Biradar J, Reddy SR, Madava A, Narayan MJ, Khurana P, Sreeman S (2013) Development and characterization of microsatellite markers for *Morus* spp. and assessment of their transferability to other closely related species. BMC Plant Biol 13(1):194

Thumilan BM, Sajeevan RS, Biradar J, Madhuri T, Nataraja KN, Sreeman SM (2016) Development and characterization of genic SSR markers from Indian mulberry transcriptome and their transferability to related species of Moraceae. PLoS ONE 11(9):e0162909

Tikader A, Dandin SB (2006) Maintenance and utilization of mulberry (*Morus* spp.) genetic resources. In: Proceedings of the reports at the International Jubilee scientific conference "Problems of maintenance and utilization of mulberry and silkworm genetic resources", Sept 25–29, 2006, Vratza, Bulgaria, pp 1–10

Tikader A, Dandin SB (2007) Pre-breeding efforts to utilize two wild *Morus* species. Curr Sci 92:1072–1076

Tikader A, Dandin SB (2008) DNA fingerprint of inter and intra-specific hybrids from *Morus* species using RAPD. Geobios 35(2–3):113–120

Tikader A, Kamble CK (2007) Mulberry breeding in India—a critical review. Sericologia 47(4):367–390

Tikader A, Kamble CK (2008) Genetic diversity of Morus species of indigenous and exotic accessions evaluated by important agronomical traits. Philip J Sci 137(1):29–38

Tikader A, Vijayan K (2017) Mulberry (*Morus* spp.) Genetic diversity, conservation and management. In: Ahuja MR, Jain M (eds) Biodiversity and conservation of woody plants, vol 17, chapter 4. Springer, Cham, pp 95–127

Tikader A, Vijayan K, Raghunath MK, Chakraborti SP, Roy BN, Pavankumar T (1995) Studies on sexual variations in mulberry (*Morus s*pp). Euphytica 84(2):115–120

Tikader A, Vijayan K, Kamble CK (2009) Conservation and management of mulberry germplasm through biomolecular approaches a review. Biotechnol Mol Biol Rev 4(4):92–104

Tipton J (1994) Relative drought resistance among selected southwestern landscape plants. J Arboricult 20(3):150–155

Toker C, Canci H, Yildirim T (2007) Evaluation of perennial wild *Cicer* species for drought resistance. Genet Resour Crop Evol 54(8):1781–1786

Tzenov PI (2002) Conservation status of mulberry germplasm resources in Bulgaria. Paper contributed to expert consultation on promotion of global exchange of sericulture germplasm satellite session of XIXth ISC Congress, September 21st–25th Bangkok, Thailand. http://www.fao.org/DOCREP/005/AD107E/ad107e01.htm

Ukaji N, Kuwabara C, Takezawa D, Arakawa K, Yoshida S, Fujikawa S (1999) Accumulation of small heat-shock protein homologs in the endoplasmic reticulum of cortical parenchyma cells in mulberry in association with seasonal cold acclimation. Plant Physiol 120(2):481–489

Uribe TF, Sanchez MD (2001) Mulberry for animal production: animal production and health paper. FAO, Rome, pp 199–202

Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTL. J Quant Trait Loci 2(1):1–5

Van Dongen JT, Licausi F (2015) Oxygen sensing and signaling. Annu Rev Plant Biol 66:345–367

Van Ooijen JW (2004) MapQTL5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen, the Netherlands

Vaucheret H, Fagard M (2001) Transcriptional gene silencing in plants: targets, inducers and regulators. Trend Genet 17(1):29–35

Vaucheret H, Béclin C, Fagard M (2001) Post-transcriptional gene silencing in plants. J Cell Sci 114(17):3083–3091

Venkateswarlu MB, Susheelamma N, Sarkar A, Datta RK (1994) Isozyme studies in mulberry germplasm introduced from Rajasthan. Indian J Sericult 33:98–99

Venkateswarlu MB, Raje Urs S, Surendra Nath B, Shashidhar HE, Maheswaran M, Veeraiah TM, Sabitha MG (2006) A first genetic linkage map of mulberry (*Morus* spp.) using RAPD, ISSR, and SSR markers and pseudo test cross mapping strategy. Tree Genet Genome 3(1):15–24

Vijayan K (2004) Genetic relationships of Japanese and Indian mulberry (*Morus* spp.) revealed by DNA fingerprinting. Plant Syst Evol 243(3–4):221–232

Vijayan K (2007) Molecular markers and their application in mulberry breeding. Intl J Ind Entomol 15(2):145–155

Vijayan K (2010) The emerging role of genomic tools in mulberry (*Morus*) genetic improvement. Tree Genet Genomes 6(4):613–625

Vijayan K, Chakraborti SP (1998) Breeding for triploid mulberry varieties in India. Indian J Sericul 37:1–7

Vijayan K, Chatterjee SN (2003) ISSR profiling of Indian cultivars of mulberry (*Morus* spp.) and its relevance to breeding programs. Euphytica 131(1):53–63

Vijayan K, Chauhan S, Das NK, Chakraborti SP, Roy BN (1997a) Leaf yield component combining abilities in mulberry (*Morus* spp.). Euphytica 98(1–2):47–52

Vijayan K, Das KK, Chakraborti SP, Roy BN (1997b) Heterosis for leaf yield and related characters in mulberry. Indian J Genet 583:369–374

Vijayan K, Doss SG, Chakraborti SP, Roy BN (1999) Genetic divergence in indigenous mulberry. Indian J Agri Sci 69:851–853

Vijayan K, Chakraborti SP, Ghosh PD (2002) Salinity induced cell membrane damage in Mulberry (*Morus* spp.). Indian J Sericult 41:70–73

Vijayan K, Chakraborti SP, Ghosh PD (2003) *In vitro* screening of mulberry for salinity tolerance. Plant Cell Rep 22(5):350–357

Vijayan K, Kar PK, Tikader A, Srivastava PP, Awasthi AK, Thangavelu K, Saratchandra B (2004a) Molecular evaluation of genetic variability in wild populations of mulberry (*Morus serrata* Roxb.). Plant Breed 123(6):568–572

Vijayan K, Srivastava PP, Awasthi AK (2004b) Analysis of phylo-genetic relationship among five mulberry (*Morus*) species using molecular markers. Genome 47(3):439–448

Vijayan K, Nair C, Chatterjee SN (2005) Molecular characterization of mulberry genetic resources indigenous to India. Genet Resour Crop Evol 52(1):77–86

Vijayan K, Srivastava PP, Nair CV, Awasthi AK, Tikader A, Sreenivasa B, Urs SR (2006a) Molecular characterization and identification of markers associated with leaf yield traits in mulberry using ISSR markers. Plant Breed 125(3):298–301
Vijayan K, Tikader A, Kar PK, Srivastava PP, Awasthi AK, Thangavelu K, Saratchandra B (2006b) Assessment of genetic relationships between wild and cultivated mulberry (*Morus*) species using PCR based markers. Genet Resour Crop Evol 53(5):873–882
Vijayan K, Chakraborti SP, Ghosh PD (2007) Foliar application of *Azatobactor chroococcum* increases leaf yield under saline conditions in mulberry (*Morus* spp.). Sci Hort 113(3):307–311
Vijayan K, Chakraborti SP, Ercisli S, Ghosh PD (2008a) NaCl-induced morpho-biochemical and anatomical changes in mulberry (*Morus* spp.). Plant Growth Regul 56(1):61–69
Vijayan K, Chakraborti SP, Doss SG, Ghosh PD, Ercisli S (2008b) Combining ability for morphological and biochemical characters in mulberry (*Morus* spp.) under salinity stress. Intl J Entomol 16(2):67–74
Vijayan K, Doss SG, Chakraborti SP, Ghosh PD (2009a) Breeding for salinity resistance in mulberry (*Morus* spp.). Euphytica 169(3):403–411
Vijayan K, Nair CV, Chatterjee SN (2009b) Diversification of mulberry (*Morus indica* var. S36), a vegetatively propagated tree species. Casp J Environ Sci 7(1):23–30
Vijayan K, Doss SG, Chackraborti SP, Ghosh PD (2010) Character association in mulberry under different magnitude of salinity stress. Emir J Food Agri 22:318–325
Vijayan K, Tikader A, Weiguo Z (2011a) Mulberry (*Morus* L.). In: Kole C (ed) Wild crop relatives: genomic & breeding resources. Springer, Heidelberg, pp 75–95
Vijayan K, Tikader A, da Silva JAT (2011b) Application of tissue culture techniques propagation and crop improvement in mulberry(*Morus* spp.). Tree for Sci Biotechnol 5(1):1–13
Vijayan K, Ravikumar G, Tikader A (2018) Breeding for higher fruit production in Mulberry (*Morus* spp.). In: Al-Khayri JM, Jain S, Johnson D (eds) Advances in plant breeding strategies: fruits, vol 3. Springer, Cham, pp 89–130. https://doi.org/10.1007/978-3-319-91944-7_3
Voesenek LACJ, Bailey-Serres J (2015) Flood adaptive traits and processes: an overview. New Phytol 206(1):57–73
Voesenek LACJ, Rijnders JHG, Peeters AJM, van de Steeg HM, de Kroon H (2004) Plant hormones regulate fast shoot elongation under water: from genes to communities. Ecology 85(1):16–27
Wang ZW, Yu MD (2001) AFLP analysis of genetic background of polyploid breeding materials of mulberry. Acta Sericol Sin 27(3):170–176
Wang H, Lou C, Zhang Y, Tan J, Jiao F (2003) Preliminary report on *Oryza* cystatin gene transferring into mulberry and production of transgenic plants. Acta Sericol Sin 29:291–294
Wang H, Tong W, Feng L, Jiao Q, Long L, Fang R, Zhao W (2014a) De Novo transcriptome analysis of mulberry (*Morus* L) under drought stress using RNA seq technology. Russ J Bioorg Chem 40(4):423–432
Wang XL, Yu YS, Yang Y, Liu CY, Li J, Yu MD (2014b) Molecular cloning and expression of a nitrite reductase gene from mulberry (*Morus* L.). Plant Cell Tiss Org Cult 121(2):301–309
Wang C, Zhi S, Liu C, Xu F, Zhao A, Wang X, Tang X, Li Z, Huang P, Yu M (2017a) Isolation and characterization of a novel chalcone synthase gene family from mulberry. Plant Physiol Biochem 115:107–118
Wang D, Dong Z, Zhang Y, Guo K, Guo P, Zhao P, Xia Q (2017b) Proteomics provides insight into the interaction between mulberry and silkworm. J Proteome Res 16(7):2472–2480
Wang D, Zhao L, Wang D, Liu J, Yu X, Wei Y, Ouyang Z (2018) Transcriptome analysis and identification of key genes involved in 1-deoxynojirimycin biosynthesis of mulberry (*Morus alba* L.). Peer J 6:e5443
Wangari NP, Gacheri KM, Theophilus MM, Lucas N (2013) Use of SSRmarkers for genetic diversity studies in mulberry accessions grown in Kenya. Intl J Biotechnol Mol Biol Res 4(3):38–44
Wani SA, Bhat MMA, Malik GN, Zaki FA, Mir MR, Wani N, Bhat KM (2013) Genetic diversity and relationship assessment among mulberry (Morus spp.) genotypes by simple sequence repeat (SSR) marker profile. Afr J Biotechnol 12(21):3181–3187
Wassenegger M (2002) Gene silencing. Intl Rev Cytol 219:61–113

Weiguo Z, Zhihua Z, Xuexia M, Yong Z, Sibao W, Jianhua H, Hui X, Yile P, Yongping H (2007) A comparison of genetic variation among wild and cultivated *Morus* species (Moraceae: *Morus*) as revealed by ISSR and SSR markers. Biodivers Conserv 16(2):275–290

Wesley SV, Helliwell CA, Smith NA, Wang M, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA, Robinson SP (2001) Construct design for efficient, effective and high throughput gene silencing in plants. Plant J 27(6):581–590

Xiang Z, Zhang Z, Yu M (1995) A preliminary report on the application of RAPD in systematics of *Morus*. Acta Sericol Sin 21:203–207

Xin Y, Ma B, Xiang Z, He N (2019) Amplification of miniature inverted-repeat transposable elements and the associated impact on gene regulation and alternative splicing in mulberry (*Morus notabilis*). Mob DNA 10(1):27

Yamauchi T, Colmer TD, Pedersen O, Nakazono M (2018) Regulation of root traits for internal aeration and tolerance to soil waterlogging-flooding stress. Plant Physiol 176(2):1118–1130

Yang X, Yang L, Zheng H (2010) Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidaemia rats. Food Chem Toxicol 48(8–9):2374–2379

Yigit D, Akar F, Baydas E, Buyukyildiz M (2010) Elemental composition of various mulberry species. Asian J Chem 22(5):3554–3560

Yokoi S, Bressan RA, Hasegawa PM (2002) Salt stress tolerance of plant. JIRCAS Work Rep 25–33

Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17(2):155–160

Yu J, Pressoir G, Briggs WH IV, Bi YM, Doebley J, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Gene 38(2):203–208

Yuan AH, Ma J, Jiang XF, Li S (2006) Screening of glucosidase inhibitors from various fractions of Mulberry leaves. Zhongguo Zhong Yao Za Zhi 31(3):223–227

Zhang J, Yang T, Li RF, Zhou Y, Pang YL, Liu L, Fang RJ, Zhao QL, Li L, Zhao WG (2016) Association analysis of fruit traits in mulberry species (Morus L.). J Hort Sci Biotechnol 91(6):645–655

Zhao W (2005) Studies on genetic diversity and molecular 1018 phylogeny of germplasm resources of genus *Morus*. Chinese Academy of Agricultural Sciences, Zhengjiang (Doctor Thesis, in Chinese). https://kns.cnki.net/KCMS/detail/detail.aspx?dbcode=CDFD&dbname=CDFD9908&filename=2005114928.nh&uid=WEEvREcwSlJHSldRa1FhcEFLUmViSGFrN2RkM3lKNEMvZUdCNS9MYm5ocz0=$9A4hF_YAuvQ5obgVAqNKPCYcEjKensW4IQMovwHtwkF4VYPoHbKxJw!!&v=MjUwOTFPcDVFYlBJUjhlWDFMdXhZUzdEaDFUM3FUcldNMUZyQ1VSN3FmWStadUZ5dmxWNzNJVjEyN0c3SzVHdGo=,

Zhao W (2008) Full-length cDNA library construction and analysis of the expressed sequence tags (ESTs) of young mulberry shoots. Postdoctoral report 8. Nanjing University, China

Zhao KF, Littlewood A, Harris PJC (1992) Responses of *Gleditsia triacanthos* seedlings to salt stress. Intl Tree Crop J 7(3):149–153

Zhao W, Miao X, Jia S, Pan Y, Huang Y (2005) Isolation and characterization of microsatellite loci from the mulberry, *Morus L.* Plant Sci 168(2):519–525

Zhao WG, Zhou ZH, Miao X, Wang S, Zhang L, Pan Y, Huang Y (2006) Genetic relatedness among cultivated and wild mulberry as revealed by inter-simple sequence repeat (ISSR) analysis in China. Can J Plant Sci 86:251–257

Zhao W, Wang Y, Chen T, Jia G, Wang X, Qi J, Pang Y, Wang S, Li Z, Huang Y, Pan Y (2007a) Genetic structure of mulberry from different ecotypes revealed by ISSRs in China: an implications for conservation of local mulberry varieties. Sci Hort 115(1):47–55

Zhao WZ, Zhihua Z, Xuexia M, Yong Z, Sibao W, Jianhua H, Hui X, Yile P, Yongping H (2007b) A comparison of genetic variation among wild and cultivated Morus species (Moraceae: *Morus*) as revealed ISSR and SSR markers. Biodivers Conserv 16(2):275–290

Zhao WG, Wang W, Yang YH, Huang YP, Pan YL (2008) Genetic diversity of mulberry local varieties from different ecotype as revealed by ISSR analysis in China. Acta Sericol Sin 34:1–5

Zhao W, Fang R, Pan Y, Yang Y, Chung J, Chung I, Park Y (2009) Analysis of genetic relationships of mulberry (*Morus* L.) germplasm using sequence-related amplified polymorphism (SRAP) markers. Afr J Biotechnol 8(11):2604–2610

Zhou H, Liu B, Weeks DP, Spalding MH, Yang B (2014) Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. Nucleic Acid Res 42(17):10903–10914

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

Zhu P, Cai Y, Yu J, Zhao A, Liang Y, Liu C, Wang C, Yu M (2017) Characterization and expression of abscisic acid signal transduction genes during mulberry fruit ripening. Acta Physiol Plant 39(7):149

Chapter 8
Genetics and Genomics of Abiotic Stress in Rubber Tree (*Hevea Brasiliensis)*

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Abstract Among various latex yielding plants, Para rubber tree *Hevea brasiliensis* is the viable source of natural rubber for commercial exploitation and which accounts for more than 98% of the world's natural rubber production. The unique physical and chemical properties compared to synthetic rubber make it indispensable and irreplaceable by any synthetic alternatives. Also, it is an environmental friendly and sustainable raw material in contrast to the environmental pollution of the industrial synthetics. Because of the economic importance of the crop, there is always a demand—supply gap. The decline in natural rubber production occurs mainly due to poor soil fertility, environmental vagaries like drought, cold, high solar radiation, low atmospheric humidity. These factors also limits the expansion of rubber cultivation to marginal areas. Taxing environment limits rubber productivity in the conventional rubber growing tracts also. As a result of abiotic stresses, ROS (reactive oxygen species) levels enhances and have a negative influence in biosynthesis of chlorophyll, photosynthetic capacity, and carbohydrate, protein, lipid, and antioxidant enzyme activities. High levels of salts or toxic metals (aluminium, arsenate, cadmium etc.) accumulated in the soil also significantly influence the growth of plants and negatively affect plant productivity. In the present scenario of unprecedented climatic conditions, combinations of different types of stresses can occur simultaneously in areas of crop cultivation worldwide, causing huge loss worth billions of dollars. Hence, importance of genetic improvement in a perennial tree crop like rubber facing the vagaries of environment needs no further emphasis. Genomic studies for understanding the molecular mechanisms behind abiotic stresses and their manipulation can solve the issues to a greater extend. This chapter gives an overview of the different abiotic stresses and genomic work carried out in rubber during the last two decades which have far-reaching impact on *Hevea* improvement towards plant health, productivity, and enhanced stress tolerance.

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8.1 Introduction

The Para rubber tree, *Hevea brasiliensis* (Muell. Arg.),is a recently domesticated tree, commercially exploited for the production of natural rubber (NR) (Fig. 8.1). It is a tropical tree species originated from the hot and humid Amazonian rain forest in Central and South America. Although, there are about 7,500 species of rubber yielding plants in tropical and subtropical regions, the para rubber tree *H. brasiliensis,* commonly known as rubber, is the prime source of NR for commercial exploitation (Greek 1991). NR, chemically known as *cis*-1, 4-polyisoprene, is extracted from the latex collected from the bark of this tree by controlled wounding or tapping. Even

Fig. 8.1 A young rubber plantation

though rubber products like shoes were made as early as 1755, it was only after the finding of vulcanization process by Charles Goodyear in 1839 (Priyadarshan and Clement-Demange 2004). NR became an industrial commodity with more than 40,000 products manufactured out of it. Rubber is cultivated commercially in more than 20 countries including Thailand, Indonesia, Malaysia and India.

There is always a gap in the demand and supply of NR which warrants an increase in production to meet the demand. Rubber has a juvenile phase of 6 to 7 years and an economic phase of about 25 years. Investment in rubber plantation has long term implications and the high initial development cost further signifies its gravity. In India, rubber is predominantly a small grower's crop and the resource-poor small holders in the industry make the investment irreversible. Once the planting is done, the growers lack the flexibility to adopt newly developed clones as they have to wait until the existing tree reaches the replanting stage, which normally takes 30 years. Owing to these rigidities, the technology adopted in the selection of planting materials has long-term implications on the yield profile of the crop as well as the income stream of growers.

The escalating demand for NR can be met either by increasing the area under cultivation or by increasing the productivity. The decline in NR production occurs mainly due to unfavorable weather conditions. Poor soil fertility and environmental vagaries such as drought, cold, high solar radiation, low atmospheric humidity, etc., limit the expansion of cultivation in major rubber producing countries. Taxing environment limits rubber productivity in the conventional rubber growing tracts also. As a consequence of deforestation and global warming, rapid and unprecedented climate changes happen in the atmosphere. A significant increase in the atmospheric CO_2 concentrations was also observed in the past two centuries. Prolonged episodes of drought, heat, cold and flood affect plant growth and yield, causing huge lossworth billions of dollars. Hence, importance of genetic improvement in a perennial tree crop like rubber facing the vagaries of environment needs no further emphasis.

8.2 Abiotic Stresses Associated with Rubber Cultivation

Because of sessile nature, plants are exposed to diverse environmental stresses such as drought, wind, cold, poor soils and presence of heavy metals, which adversely affect plant growth and yield. In the present scenario of unprecedented climatic conditions, combinations of different types of stresses can occur simultaneously in areas of crop cultivation worldwide. As a result of abiotic stresses, ROS (reactive oxygen species) levels enhances and have a negative influence in biosynthesis of chlorophyll, photosynthetic capacity, carbohydrate, protein, lipid, and antioxidant enzyme activities. High levels of salts or toxic metals (aluminium, arsenate, cadmium etc.) accumulated in the soil also significantly influence the growth of plants and negatively affect plant productivity.

8.2.1 Cold Stress

Cold induced damage to rubber tree is a complex phenomenon affecting growth and vigor of plants as well as productivity of crop. In low temperature (0–10 °C) areas such as South Central China and North Eastern states of India, rubber cultivation and latex production is strongly affected by cold stress (Priyadarshan et al. 2005). Severe inhibition of growth at 20 °C or less throughout the year was reported in rubber (Jiang 1988). During the early stages of establishment, rubber plants suffer from cold stress when exposed to low temperature (Ailiang 1984; Meenattoor et al. 2000) causing growth reduction and resulted in yield loss in NE regions of India (Sethuraj et al. 1989; Alam et al. 1998; Jacob et al. 1999a, b) and China (Zongdao and Yanguing 1992). Generally, in North Konkan and West Bengal, the stress season receives high intensities of solar radiation and long sunshine hours (Devakumar et al. 1999; Dey et al. 1999; Jacob et al. 1999a, b). Alam et al. (2003) reported that the yield reduction as a consequence of winter was less in RRIM 600 compared to PB 235. Among elite clones, RRIM 600 and PB 235 performed better in growth and yield in cold stress prone North East (Vinoth et al. 1996; Meenattoor et al. 1991; Alam et al. 1998; Mondal et al. 1999). During cold season, significant clonal variation was observed and leaf photosynthesis was low resulting in poor biomass increment and relative growth rate (Meti et al. 2003) and caused leaf necrosis affecting tree development and latex production (Mai et al. 2010). The clones PR 261, RRIC 104 and RRII 208 showed higher yield during winter, and the clones Haiken 1, RRII 208 and PB 260 were also found to be performing better under low temperature (Sandeep Kumar et al. 2015).

8.2.2 Drought and Heat Stress

Owing to the origin of rubber tree from the hot and humid Amazonian river basin, a well distributed annual rainfall ranging from 2000 to 4000 mm, hot and sunny days with a temperature range of 21–35 ℃ and relative humidity (RH) of 80% or above are considered the best growth conditions (Verheye 2010). In India, Kerala and Kanyakumari district of Tamil Nadu are considered as the traditional rubber growing regions while North Konkan, parts of Karnataka, Odisha, Madhya Pradesh and low temperature prevailing areas of North-Eastern states are considered as the non-traditional belt. Unlike traditional regions, marginal and non-traditional dry areas, during summer season, are constantly confronted with various agro-climatic constraints. Soil and atmospheric drought, higher atmospheric temperature concomitant with high solar light and low relative humidity have been reported as major constraints limiting the establishment of rubber cultivation in such areas (Jacob et al. 1999a, b; Alam et al. 2005). These stress phenomena adversely affected performance of the crop and rubber yield (Chandrasekhar et al. 1990; Jacob et al. 1999a, b). The detrimental effect of drought stress on growth and productivity of the crop have been confirmed by

several authors (Sethuraj 1986; Ouseph 1987; Sethuraj et al. 1989; Chandrasekhar et al. 1990; Bhaskar et al. 1991; Mohanakrishna et al. 1991; Wichichonchai and Manmeun, 1992; Manmuen et al. 1993; Rao et al. 1998) and increased TPD incidences leading to death at severe drought conditions (Huand and Pan 1992). However, during summer season, by irrigation, rubber can be successfully grown in this region (Sethuraj et al. 1989; Bhaskar et al. 1991, 1999; Vijayakumar et al. 1998). Water stress and atmospheric water vapour deficit inhibited the latex flow (Paaradekooper and Sookmark 1969, Buttery and Boatman 1976; Sethuraj et al. 1984; Pakianathan et al. 1989; Rao et al. 1990; Dey et al. 1999) and severe drought stress decreased rubber yield by reducing the total volume of latex (Rao et al. 1988; Jacob et al. 1999a, b). In recent past, even in the traditional areas in India, drought is common affecting early establishment of rubber cultivation (Satheesh and Jacob 2011; Jessy et al. 2014) and countries like Sri Lanka and Thailand are also experiencing similar trend (Samarappuli and Yogaratnam 1998; Chantuma et al. 2012). This is really a major threat for rubber cultivation for the upcoming years, because of the unpredicted climate change and global warming. In this context, identification of drought tolerant clones and breeding for elite high latex rubber clones with enhanced tolerance to drought is need of the day.

8.2.3 Flooding and Submergence

Since rubber tree is a flooding intolerant species, literature available in relation to flooding tolerance is scanty. A significant reduction in the girth of the tree in response to prolonged flooding was reported as early as 1988 in rubber tree as in any other flood-intolerant species (Sena Gomes and Kozlowski 1988). However, no change in stomatal activity was observed.

8.2.4 Soil Acidity and Related Stress

Fifty percent of potentially arable lands throughout the world is affected by soil acidification, particularly in tropical and subtropical zones. Improper farming practices and environmental problems, including acid rains gradually exacerbates soil acidity. In India, the soil is acidic in about 30% of cultivated area. The primary cause of poor soil fertility is the toxicity of metal elements and the loss of nutrients. The soil acidity has been associated with hot humid climate and heavy precipitation and when the pH is less than 5.5 and aluminium (Al) saturation is high. Al toxicity is the most common cause of acid soil infertility. When Al concentration was 15 ppm or more in the solution, the growth of the rubber plants was affected and the toxic effect was seen in the roots where the root tip and lateral roots became thickened and turned brown. However, less than 15 ppm of Al, the rubber plants were growing very well (Bueno et al. 1988). The level of exchangeable Al^{3+} in the surface soil (0–25 cm depth)

ranged from 0.36 to 0.98 Cmol (+)/kg and high Al concentration in the soil was harmful to the roots and it affects translocation of calcium and phosphorous (Suresh et al. 1994). When rubber seedlings treated with $AlCl_3$ at a concentration higher than 200 mmol·L^{-1} cell membrane permeability, free proline content, and soluble sugar content were significantly increased, while the relative water content, catalase and peroxidase activities, chlorophyll (Chl) a content, Chl maximum fluorescence yield, maximum photosynthesis efficiency and potential activity of photosystem (PSII), photochemical quenching coefficient, non-photochemical quenching coefficient and photosynthetic electron transport rate were significantly decreased. However, lower than 100 mmol·L^{-1}, the aforesaid parameters showed no significant variation indicating that the rubber tree could tolerate some degree of Al toxicity and threshold concentration would be between 100 to 200 mmol·L^{-1} (An et al. 2018). In acid soil, phosphorus (P) fixation is high due to the presence of large proportion of iron (Fe) and Al oxide and hydroxides (Karthikakuttyamma et al. 1991). Soils in the traditional rubber growing belt are mainly red ferruginous dominated by Fe and Al oxides and hydroxides and kaolonite clay. These soils are low base status soils with consequent higher acidity (Karthikakuttyamma et al. 2000). Al toxicity can be corrected by liming to a pH of 5.5 to 6.0 for precipitating exchangeable Al as Al hydroxide. Also the near neutral pH improves the availability of almost all nutrients to crops. Liming significantly increased the availability of calcium and reduced that of K and Mg. Liming in combination with fertilizer application significantly improved the availability of P, potassium (K) and calcium (Ca) in the soil (Syamala et al. 2003). The average rainfall is another determining factor for base saturation percent and exchangeable bases. Low base saturation and low status of K, Ca and magnesium (Mg) is the characteristic of high rainfall areas (Joseph 2011). Rubber is performing well in strongly acidic pH but soils with extremely acidic pH along with higher exchangeable Al and H^+ ions concentration retard the growth. At extremely low pH (4.4) the availability of absorption is limited by the high P fixation (Ulaganathan et al. 2005). With continuous cultivation of rubber, there is a shift in the soil pH from strongly acidic to extremely acidic (Joseph 2016). Shafar et al. (2017) reported that when pH value reaches 5.0, the Al in the soil solution undergoes precipitation and becomes less toxic. The soil pH and base nutrient status significantly influenced the growth of rubber seedlings. When soil conditions especially soil pH, Al^{3+} and H^+ ions' contents exceed the limit, the growth of rubber was affected (Ambily and Joseph 2018).

The growth of rubber is greatly influenced by soil physical, chemical and mineralogical properties (Kharche et al. 1995). Uncertain rainfall pattern has been reported to increase causality of young plants immediately after planting (Jessy et al. 2011). High temperature and changes in the amount or pattern of rainfall caused changes in soil CO_2 emission thereby depleting the soil organic matter status which determines the soil fertility (Abraham and Jessy 2018). Drought tolerant RRIC 100 series of clones were superior in growth and they showed high levels of K in leaf and bark under stressed condition (Samarappuli et al. 1992) and application of high levels of K, the young rubber plants are overcoming moisture stress (Samarappuli et al. 1993). K is a major contributor to solute accumulation for rubber clones with high

osmoregulation during water stress (Karyudi 2004). In the drought tolerant 400 series clones as well as germplasm accessions, the level of leaf K content was high during pre-stressed and stressed condition (Ambily et al. 2020).

8.2.5 Salinity Stress

Very little information is available regarding the salinity stress in rubber. Salinity stress adversely affects the root-to-shoot ratio, seedling vigor and growth of rubber (Zeng et al. 2007). Dehydrins identified from *Hevea*, designated as *HbDHN1* and *HbDHN2* genes were overexpressed in Arabidopsis. Overexpressed lines showed increased tolerance to salt, drought, and osmotic stress. The transgenic plants had more proline accumulation, reduction in electrolyte leakage, higher water content, higher antioxidant activity and less accumulation of hydrogen peroxide and superoxide (Cao et al. 2017).

8.2.6 Water Use Efficiency and Root Characters

The amount of carbon assimilated as biomass or grain produced per unit of water used by the crop is denoted as water use efficiency (WUE). Water use efficiency is a determining factor in plant growth and yield. WUE at the whole-plant level is an indicator of both the water-use strategy of the plant, and the ecophysiological processes linking the carbon and water cycles. Proper crop management methods can optimize crop production with limited water supply. It was observed that water mining rate of rubber trees is mainly controlled by available soil moisture and prevailing climatic condition. In North East Thailand also, a decline in water consumption of rubber tree was observed as the dry season progressed (Isarangkool Na Ayutthaya et al. 2009). It is generally believed that when mature rubber trees are felled water levels in the nearby wells go up. The published information indicated that water mining rate in rubber is modest compared to several forest and cultivated tree species. In the case of rubber, the transpiration rate of mature tree was estimated to be 20–25 L water/tree/day or the range of 1.5–2.0 mm per day (Annamalainathan et al. 2013). In 2020, Annamalainathan et al. compared water mining by rubber trees grown under three different agro-climatic conditions viz. traditional, hot and dry North Konkan region, cold prone area in West Bengal. The mean water mining rate was 22 L/day/tree in Kerala, 23 L/tree/day in West Bengal and 25 and 19 L/tree/ day for irrigated and rainfed trees in Maharashtra. Water use efficiency of mature rubber tree estimated as dry rubber production or tree biomass for a unit amount of water consumed varied between three regions. In Kerala, this was 8.0×10^{-4} kg rubber kg^{-1} water and 3.8×10^{-3} kg water where in Maharashtra WUE was very less (3.7×10^{-4} kg rubber kg^{-1} water and $2.0\ 8 \times 10^{-3}$ kg biomass kg^{-1} water in rainfed and 4.8×10^{-4} kg rubber kg^{-1} water and 2.3×10^{-3} kg biomass kg^{-1} water in irrigated trees). In West

Bengal, WUE was 6.7×10^{-4} kg rubber kg^{-1} water and 3.4×10^{-3} kg biomass kg^{-1} water. Results showed that higher the rubber yield or biomass production, the higher the WUE and therefore, WUE of rubber tree predominantly affects rubber yield or biomass rather than by amount of water consumed. In *Hevea*, xylem vulnerability to cavitation of branches showed no variation among clones, while it was observed in petioles. Clonal differences were also observed in stomatal response and in leaf shedding behavior in response to a stimulated drought (Jinagool et al. 2015). Lin et al. (2018) studied dynamics of WUE and its relationships with environmental and biological factors in rubber plantations in Southwest China and opined that, the factors affecting carbon sequestration may be regulating WUE rather than water consumption.

8.3 *Hevea* Genetic Resources

The progress of conventional breeding relies on the available genetic resources in the species. The genus *Hevea* includes 11 species: *H. brasiliensis, H. guianensis, H. benthamiana, H. pauciflora, H. spruceana, H. microphylla, H. rigidifolia, H. nitida, H. camporum, H. camargoana* and *H. paludosa* (Ule 1905; Schultes 1990). According to Clement-Demange et al. (2000), effective cross pollination is possible among the 10 species of *Hevea* since there is no species barrier among them. Generally, *Hevea* can be considered as a species complex (Gonçalves et al. 1990; Priyadarshan and Gonçaalves 2002). *Hevea* species exist naturally in South American countries including Peru, Brazil, Bolivia, Venezuela, Ecuador, Surinam, Colombia and French Guiana. *Hevea* species generally have a diploid chromosome number $2n = 36$. However, few exceptions with one triploid clone from the species of *H. guianensis* with 54 chromosomes and one genotype of *H. pauciflora* with 18 chromosomes were also reported (Baldwin 1947; Majumder 1964). Additionally, an amphidiploid behavior was also noticed for *H. brasiliensis* (Ramaer 1935; Ong 1975; Wycherley 1976). It is a well-known fact that all presently cultivated high yielding clones of *H. brasiliensis* were originated from a few seedlings and have a very narrow genetic base (Dijkman 1951).

According to Wycherley (1968), 70,000 seeds transferred from Brazil to England by Henry Wickham in 1876 paved the way for the introduction of rubber trees to Asia. About 2,397 seedlings were sent to various Asian countries like Sri Lanka, Java and Singapore between 1876–1877 (Wycherly 1968; Dean 1987; Baulkwill 1989). The 22 introduced seedlings were planted in the Singapore botanical garden and this became the main source of the rubber trees planted in Asian countries. This collection was later called as Wickham collection and was the foundation for rubber domestication during twentieth century. Today the rubber tree has become an important perennial crop as the major source of commercial rubber in the world (Lam et al. 2012; Saha and Priyadarshan 2012).

Another major genetic resource of rubber which is being utilized for breeding by rubber breeders around the globe is the native (wild) Amazonian population. It is

a collection of wild accessions from the Amazonian forest, which are undergoing evolution and have not been subjected to modification by human selection. Expeditions for the identification, collection and bringing together of allied species and Amazonian accessions were initiated as early as 1890. Majority of them are low yielders with an average yield less than 20% of Wickham clones (Clément-Demange et al. 2001).

Since 1890, several expeditions were conducted to the Amazon basin for the collection and transfer of different *Hevea* species and accessions at the individual as well as organizational levels. During 1951–1952, seedlings of five *Hevea* species (*H. brasiliensis, H. guianensis, H. benthamiana, H. spruceana*, and *H. pauciflora*) were introduced to Malaysia (Tan 1987). Eleven clones each of *H. brasiliensis*, and *H. benthamiana* along with105 hybrid materials were imported by Sri Lanka during 1957–1959 in collaboration with USDA, Instituto Agronomico do Norte (IAN) (Brazil), and Liberia. Subsequently Malaysia received many of the clones from Sri Lanka (Tan 1987). CNRAin Côted'Ivoire, in cooperation with CIRAD introduced 53 accessions including 10 accessions from allied *Hevea* species from different centers like French-Brazilian collection from Acre and Rondonia, Firestone collection in the Madrede Diosbasinin Peru (MDFaccessions) and Brazilian Research Center, EMBRAPA, in Manaus. In addition, 302 accessions from Calima site and 41 accessions from Palmirasite were rescued from the collections made by R.E. and transferred to Côted' Ivoire in 1987. Collections from Brazil (mostly from Rondonia) were active until 1982 (Goncalves et al. 1983).

International Rubber Research and Development Board (IRRDB), the research and development network of rubber research institutes of rubber producing countries had taken initiative and conducted an expedition to the Amazon river basin during 1981 for collecting seeds and bud-woods from elite trees with the intention of expanding the available gene pool of rubber. They collected 63,768 seeds, 1,160 seedlings and 1,413 m of bud-wood from elite trees mainly from Acre, Rondonia, and Mato Grosso states (Tan 1987; Simmonds 1989). The two IRRDB germplasm centers established in Malaysia and in Côte d'Ivoire using these collections served as the supplier of these material to member countries.

With the intention of introgressing more variation and novel potential alleles to the Wickham clones, hybridization between Wickham and Amazonian accessions were carried out by research organizations like CIRAD, CNRA and Rubber Research Institute of India (RRII). RRI of India used two Wickham clones, RRII 105 and RRIM 600 as females along with seven wild accessions as males. Among the hybrid progenies developed, five showed better performance than the RRII105 along with other superior secondary attributes. A yield increase of 14–82% was reported (Sankariammal and Mydin 2011) in preliminary trials.

Attempts for the evaluation and utilization of Amazonian accessions by Côte d'Ivoire and France (CIRAD and CNRA) revealed that the wild Amazonian collections evaluated are far inferior in terms of yield (200–300 kg/ha) compared to Wickham clones. The average latex yield in Wickham x Amazonian crosses were also found to be low in their studies (Saha and Priyadarshan 2012). Similar evaluation and introgression programs are being conducted by most of the rubber growing

countries and it is anticipated that their collective efforts will substantially contribute towards the improvement of traits of interests like increased yield, disease resistance and tolerance to abiotic stresses.

8.4 Glimpses on Classical Genetics and Traditional Breeding

8.4.1 Traditional Breeding Methods

The intension of genetic improvement of rubber is to bring together desirable traits from a genetically variable base population and to fix them. Rubber tree is a cross pollinated species which is also amenable to vegetative propagation through bud-grafting. The different breeding methods followed conventionally are described below.

8.4.1.1 Mother Tree (Plus Tree) Selection

Use of unselected seedlings as planting materials was the initial practice followed for rubber cultivation. Later, choice of selected seeds from healthy trees became the practice. In India, ortet selection was initiated in 1954 realizing the importance of indigenously developed planting materials and this is the oldest breeding method in rubber. It involves systematic screening of large plantations for the identification of elite trees, their vegetative multiplication and subsequent field evaluation and selection of outstanding genotypes. Historically, the base populations of rubber breeding in Asia were mother trees or ortets selected from extensive populations of seedlings in commercial plantings (Ho et al. 1979; Tan 1987). A tangible number of early primary clones like GL 1, PB 28/59, BD 10, AVROS 255, Tjir1, PR 107, GT 1, Mil 3/2, and Hil 28 were developed in Indonesia, Malaysia and Sri Lanka through ortet selection. A yield improvement of 150% over the original unselected population was reported for many of these clones (Khoo et al. 1982). The primary clones like Pil D65, GL 1, Pil B84, PB 86, PB 6/9,PB 56 and Tjir 1 were used as parents for developing improved clones (Saha and Priyadarshan 2012). Jacob et al. (2013) observed that some of the very high yielding clones cultivated world over is either hybrids of a few early primary clones like GT 1 and Tjir 1 from Indonesia and PB 86 and GL 1 from Malaysia or crosses between hybrids developed from them.

8.4.1.2 Hybridization and Clonal Selection

The most successful traditional breeding method in rubber is hybridization and clonal selection. With the vegetative propagation system the desirable genotypes can be

fixed easily. Tangible number of high yielding hybrid clones were developed by all the major rubber growing countries and they showed good performance not only in the country of origin but also in countries outside they were bred. During initial years, early primary clones were used as parents in the hybridization program. According to Simmonds (1989) rubber breeders followed a cyclical generation-wise assortative mating (GAM) wherein the best clones in each series were further used as parents in subsequent series. Rubber Research Institute of Malaysia developed RRIM 500 to 1000 series with superior yield than their parental clones. Meanwhile Prang Basar Institute in the private sector in Malaysia, selected a series of PB clones of commercial significance. PR, AVROS, BPM, LCB, PPN and RR clones developed by the Indonesian Research Institute, the RRIC clones originated from Sri Lanka, KRS clones from Thailand and Haiken, YRITC and SCATC clones from China were generated from hybridization and clonal selection. Hybridization programmes were initiated in India by 1954 and consequently RRII 100, 200 and 300 series clones were developed (Annamma et al. 1990). From the RRII 100 series, RRII 105 is a high yielding one with better adaptation to the traditional rubber belt in India and occupied more than 90% of the cultivated area till recently. Later, five elite clones of the RRII 400 series were generated through hybridization and selection (Licy et al. 2003). Among this, RRII 430 is a superior performer in terms of yield, growth and yield stability, along with high level of tolerance to abiotic (drought) and biotic (Coryneospora leaf disease and Abnormal Leaf Fall (ALF) stresses (Mydin 2014).

8.4.1.3 Breeding Without Breeding (BwB)/Polycross Breeding

A concept of breeding without breeding was introduced by El-Kassaby and Lstibůrek (2009) which involves the collection of full sib and half sib seeds originated from natural pollination from an orchard with a collection of elite cultivars or clones, circumventing the artificial cross pollination. This can be used for both breeding fullsib (FS) offspring and seedlings generated from polyclonal seeds. A polyclonal garden or a clone evaluation garden planted with completely randomized design (CRD) can be used. A well-organized breeding orchard with the required parental combinations are planted to ensure the recovery of maximum number of recombinants which can generate considerable number of promising progenies. There are recent reports in support of this concept (Mydin 2011; Mydin et al. 2016; Mydin 2019). Progenies can be evaluated and the best performers can be selected. The designing and planting of the orchards need more care and has to be done according to the breeding objectives such as yield and biotic/abiotic resistance.

8.4.2 Achievements Through Traditional Breeding

The realization of high yield potential of bud-grafted clones compared to genetically improved seedlings accelerated the development of high yielding hybrid clones

and this gradually became the global mandate of breeding in rubber for achieving improved productivity. Significant enhancement in average rubber yield from 300 to 1600 kg/ha was achieved through conventional breeding (Tan 1987; Simmonds 1989). As a result of rigorous breeding and selection for 70 years, high performing clones like RRIM 600, RRIM 712, RRIM 501, PB 235, PB 260, PB 217, RRII 105, RRII 430, RRII 414, RRIC 100, IRCA 230, IRCA 18, IRCA 331, and BPM 24 were derived and further enhancement in yield upto 2500 kg/ha was also attained (Clément-Demange et al. 2001; Priyadarshan 2003; Saha and Priyadarshan 2012). Approximately ten-fold productivity improvement (300–3000 kg/ha/year) through the development of hybrids is very unique in the history of any perennial tree species (Varghese and Mydin 2000).

8.4.3 Limitations of Traditional Breeding and Prospect of Genomic Designing

Rubber is predominantly a small grower's crop in all the major rubber growing countries (Priyadarshan and Clement-Demange 2004). It was demonstrated that improving elite planting materials with increased yield and secondary traits is the major objective of rubber breeding. Characters including high initial vigor and high growth rate for attaining early tappability, smooth thick bark with a good laticiferous system, efficient bark renewal, and tolerance to abiotic and biotic stresses, tapping panel dryness, good response to stimulation and low frequency tapping are other criteria for selection of desirable clones. In the wake of global warming and climate change achieving these goals is gaining research priorities.

Rubber tree starts flowering about 4–5 years after planting. The plant has long gestation period of 7–8 years for tapping and again 3–15 years for assessment of performance in terms of yield and secondary attributes. Hence, the release of a clone starting from hand pollination and raising seedlings has to undergo various stages of evaluation and selection which takes more than three decades. The major bottlenecks associated with *Hevea* breeding are (1) perennial nature and long breeding and selection cycle, (2) narrow genetic base, (3) asynchronous flowering among clones, (4) low fruit set, (5) susceptibility to biotic and abiotic stresses, (6) seasonal variations in yield, (7) absence of reliable early prediction methods and (8) high genotype x environment interaction (Tan 1987).

Classical *Hevea* breeding made significant strides in augmenting its productivity. Still, the advancement made with respect to quantitative traits is limited because of the lack of understanding about the complex biochemical pathways and their interactions under different stresses. In the new century, there has been an escalating trend in the acquisition of genomic data and hence the researchers are better equipped for understanding the complex biological processes. A combination of the available genomic techniques which could generate gene sequences, DNA markers, linkage

maps, expressed sequence tags (ESTs) etc., with bioinformatics tools, will aid in developing desirable clones.

8.5 *Hevea* Genomics and Proteomics

8.5.1 Molecular Markers and Its Utilization

Molecular markers can play a crucial role in delineating the genetics of rubber tree population and germplasm lines. DNA fingerprinting using restriction fragment length polymorphism (RFLP) and ribosomal DNA variations were used mostly to assess the genetic variability among the various genotypes of *H. brasiliensis* rather than using them for marker-assisted selection (MAS). In rubber, many attempts have been made to use random amplified polymorphic DNA (RAPD), RFLP, microsatellite or simple sequence repeat (SSR) and single-stranded conformation polymorphism (SSCP) markers for clone identification and genetic variability studies (Besse et al. 1994; Luo et al. 1995; Varghese et al. 1997; Venkatachalam et al. 2002; Lekawipat et al. 2003; Roy et al. 2004; Mathew et al. 2005; Saha et al. 2005).

Conventional genetic analysis in rubber is cumbersome and time consuming because of its perennial nature, long breeding cycles and difficulties in raising F_2 progenies. Plant molecular breeding approaches were initiated primarily to address these problems so that the long breeding cycle of rubber could be cut short enabling the faster release of better performing clones to the farmers. Application of these advanced genome analysis techniques basically requires data on molecular markers and their associated traits. With the advent of next generation sequencing (NGS) technologies, a sudden spike in the rate of information generated on molecular markers including SSRs, single nucleotide polymorphisms (SNPs,) and ESTs were available to plant breeders. This vast amount of information contributed immensely towards the better understanding of the genetic diversity and population structure of rubber which would enable devising strategies for MAS of improved varieties with desired traits and facilitates the concept of breeding by design in rubber. In order to apply molecular tool for breeding in rubber, the availability of a saturated genetic map is very essential so that the genomic regions harboring major genes and quantitative trait loci (QTLs) that control important agronomic traits can be easily tagged. Genetic mapping of rubber to create dense SSR map commenced since 1996 for many important agronomic traits including QTLs for South American Leaf Blight (SALB) resistance (obtained from different mapping projects) (Seguin et al. 1996; Lespinasse et al. 2000; Le Guen et al. 2011a, b) and latex yield and growth (Clement-Demange et al. 2006, 2008). The development of NGS technologies provided ample opportunities to extract large number of SNP markers without the need of a reference genome sequence. The advantages of NGS are higher sensitivity to detect low-frequency variants, faster turn around time for sample volumes, comprehensive genomic coverage, higher throughput with sample multiplexing, and ability to sequence hundreds to

thousands of genes or gene regions simultaneously (Shendure and Ji 2008; Schuster 2008). SNPs identified using NGS techniques also facilitated construction of high density (10 SNP loci belonging to 5 loci) genetic maps (Pootakham et al. 2011; Mantello et al. 2014; Pootakham et al. 2015).

Genetic linkage map describes the linear order of markers whether genes or any other small DNA sequences in their respective linkage groups depicting their relative chromosomal locations by their pattern of inheritance (Priyadarshan 2017). It enhances our understanding on specific segments of the genome associated with a trait. Based on segregation analysis, Lespinasse et al. (2000) assembled the first ever saturated genetic map of *Hevea* encompassing 717 loci (covered 18 chromosomes) which eventually served as a reference genetic map. This was followed by various studies that focused on QTLs with amplified fragment length polymorphism (AFLP) markers for yield (Rosa et al. 2018; An et al. 2019) and growth traits (Souza et al. 2013). Identification and genotyping of common SNPs became possible by the invention of genotyping-by-sequencing (GBS), a technique which combined the NGS, genome complexity reduction techniques and barcoding. Using this technique, Pootakham et al. (2015) constructed genetic linkage maps of two populations comprising 1704 and 1719 markers with coverage of 2041 cM and 1874 cM, respectively.

A high density genetic map constructed from 149 progenies of RRIM 600 and RRII 105, generated 12,326 SNPs from 4,244 contigs with an average marker density of 1.90 cM (Shearman et al. 2015). Since the beginning of rubber cultivation, attaining higher yield potential had been the major breeding objective through improving the yield traits like trunk biomass, bark thickness and number of latex vessel rows, etc. (Priyadarshan 2003).

The first ultra-high density genetic linkage map constructed by Xia et al.(2018) revealed 17 most reliable QTLs for dry yield. The additionally found QTLs associated with defense mechanisms, energy metabolism and rubber biosynthesis pathways in by this study also revealed significant association with dry yield. In a study by Chanroj et al. (2017) two SNP markers (SNP7772 and SNP14857) associated with yield and one SNP marker (SNP53285) associated with stem growth were identified. The first ultra-high density genetic linkage map in rubber was constructed by Xia et al. (2018) which identified 17 QTLs for dry latex yield (DLY) along with promising QTL candidate genes such as thioredoxin h, plastin-like protein, calmodulin binding protein, cytochrome c oxidase and methylglutaconyl-CoA hydratase that have significant association with yield. QTL mapping for stem diameter, tree height and number of whorls was reported from a sibling population of GT1 x RRIM 701 cross (Conson et al. 2018). This study could identify key dominant genes associated with growth of rubber under water stress conditions which can be best used for MAS for drought tolerance. The recently developed ultra-high density genetic linkage maps along with the previously developed saturated genetic linkage maps can provide details on genome-wide EST-SSR markers, SNP markers and genomic regions containing major genes and for QTLs containing agronomically important traits for further molecular breeding through MAS. Since the traits like latex yield and stem growth are complex and are polygenically controlled (Simmonds 1989; An

et al. 2019) conditional QTL mapping was attempted in rubber for the first time to dissect the genetic interrelationship between stem growth and latex yield and identified several interlined QTLs for both the traits. More studies are being carried out in this aspect as well as on QTLs for biotic and abiotic tolerance. Such data could serve as precious resources for the breeders to employ in MAS programs in rubber. Details of published genetic linkage maps of rubber is provided in Table 8.1.

8.5.2 Hevea *Whole Genome Sequence Initiatives*

Prior to the formation of genome database, researchers worldwide have been isolating and identifying genes from different rubber clones. These are stored as raw data in the NCBI nucleotide database. A simple search typing '*Hevea brasiliensis*' on the search bar of NCBI nucleotide web page yields a staggering result of 2,43,820 genes, from various clones including nucleus, chloroplast and mitochondria. The rubber tree genome is distributed over 18 chromosomes (Leitch et al. 1998) and the haploid genome was estimated to be approx. 2.15 Gb (Bennett and Leitch 1997).

In 2013, Centre for Chemical Biology, University Sains Malaysia, Malaysia published the first ever draft whole genome sequence for *H.brasiliensis* from the Malaysian clone RRIM 600 (Rahman et al. 2013). They used sequence data of whole genome shotgun (WGS) generated from Roche 454/Illumina and SOLiD platforms and the final genome assembly was based on 27.86 Gb data (13 × coverage). After filtering out the repeats, the data resulted in scaffolds of a total 1119 Mb with an N50 of 2972 bp. Based on 154 microsatellite markers reported by Guen et al. (2011a, b), they further anchored 143 scaffolds and associated 1,325 genes onto 18 linkage groups of rubber. Of the total genome assembled, 72.01% was identified as repetitive DNA, which is estimated to represent 78% of the genome. Less than 2% of the total repeat elements are DNA transposons. Among them, 46.15% of total repeats are long terminal repeats (LTR)—most abundant sub-types being *Gypsy* (38.2%) and *Copia* (7.38%). The average gene, exon and intron lengths are 1332 bp, 238 bp and 332 bp, respectively. Apart from 9,767 protein-coding genes, 729 tRNA genes were identified including 12 suppressor tRNAs, 32 pseudogenes and 4 with undetermined functions. Additionally, 113 copies of 5S, 18 copies of 5.8S, 11 copies of 18S and 21 copies of 28S rRNA genes were identified in the assembled genome.

In 2016, the research group from Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences (CATAS), reported genome assembly of elite Chinese cultivar Reyan7-33-97 (Tang et al. 2016). This was based on sequence data from WGS and pooled bacterial artificial chromosome (BAC) clones and this approach extracted much more genomic data than the previous attempt by the Malaysian team. The genome assembly was based on 138 Gb (94 × genome coverage), a striking difference from RRIM 600 clone coverage. The estimated genome size of Reyan 7-33-97 clone is 1.46 Gb. Assembling data from pooled BAC clones, yielded 1.37 Gb genome assembly covering 93.8% of estimated genome size that contained 7,453 scaffolds (N50 = 1.28 Mb) as well as 84,285 contigs (N50 = 30.6 kb). They also

Table 8.1 Details of published genetic linkage maps of *Hevea brasiliensis*

References	Map length (cM)	LG number	Markers							
			Isozyme	RFLP	AFLP	RAPD	SSR	SNP	Total	Average marker distance (cM)
Lespinasse et al. (2000)	2,144	18	10	301	388	–	18	–	717	3
Rattanawong et al. (2008)	2075	18			198		229		427	4.86
Feng et al. (2010)	1,937.06	18	–	–	–	–	91	–	91	21.29
Souza et al. (2011)	2,471.20	23	–	–	–	–	225	–	225	11
Le Guen et al. (2011a, b)	2,441	18	–	–	187	–	195	–	383	6.37
Triwitayakorn et al. (2011)	842.9	23	–	–	–	–	97	–	97	11.9
Souza et al. (2013)	2688.8	23	–	–	–	–	284	–	284	10
Pootakham et al. (2015)	2,052	18	–	–	–	–	–	2,321	2,321	0.89
Shearman et al. (2015)	4,160	18	–	–	–	–	–	2186	2186	1.9
Conson et al. (2018)	3,779.70	18	–	–	–	–	224	855	1,079	3.5
Xia et al. (2018)	2,094.10	18	–	–	–	–	–	6,940	6,940	0.3
Rosa et al. (2018)	3525	23					354	151	505	7.4
An et al. (2019)	2,670.27	18	–	–	–	–	–	4543	4543	0.59

aligned all *Hevea* DNA sequences, ESTs and protein sequences available in the public domain to show mapping rates of 91.5, 97.9 and 100%, respectively. Similar to the repeats in RRIM 600, here also 71% of the genome assembly was covered by the dominant class of transposons, LTR with *Gypsy* and *Copia* being the most abundant types. In addition to finding 84,241 unique transcripts and 43,792 protein-coding genes, they also predicted 167 ribosomal RNAs, 591 miRNAs, 10 SPPRNAs, 3,445 SnoRNAs, 4 tmRNAs, 697 tRNAs, 219 snRNAs and 217 other types of RNAs.

The genome assembly of RRIM 600 was later updated by the Malaysian team and the genome was assembled based on ~155-fold combined coverage with Illumina and PacBio sequence data that revealed a total length of 1.55 Gb with 72.5% comprising repetitive DNA sequences. A total of 84,440 high-confidence protein-coding genes were predicted (Lau et al. 2016). In 2017, a research team from Thailand published a de novo hybrid genome assembly of the BPM24 rubber clone. It was subsequently scaffolded using a long-range "Chicago" technique to obtain the best assembly of 1.26 Gb (N50 = 96.8 kb). Using a SNP based genetic map, 28.9% of the genome assembly (approx.363 Mb) was successfully anchored into rubber tree's 18 linkage groups (Pootakham et al. 2017).

The most recent genome published is of the elite cultivar GT1 by Liu et al. (2020) using single-molecule real-time sequencing (SMRT) and Hi-C technologies. The chromosome-based mapping technology generated clean sequence datasets of 348.14 Gb and yielded approximately 261.4-fold coverage. The genome size was estimated to be 1.56 Gb and the genome heterozygosity was estimated to be 1.60%–1.62%. The contig and scaffold N50 lengths were 8.79 kb and 31.3 kb, respectively. Resolving the repetitive structure and heterozygous regions from previous genome assemblies (Rahman et al. 2013; Lau et al. 2016; Tang et al. 2016; Pootakham et al. 2017), they generated a total of 161.86 Gb (103.75-fold sequence coverage) of long-read sequence data from 20-kb and 40-kb insert libraries with subread N50 lengths of 9.07 kb and 18.34 kb, respectively. A PacBio-only assembly was also performed using an overlap layout-consensus method and obtained a 1.47 Gb genome assembly with a contig N50 of 152.7. This final assembly of the rubber tree genome comprises 16 023 scaffolds, of which 15 885 scaffolds (>10 kb) represent 99.93% of the 1.47-Gb genome. A final reference genome of the rubber tree was obtained on the chromosome level by anchoring 1442-Mbp-sized contigs into 18 chromosomes. The total length of the assembled genome sequences accounts for 92.4% of the estimated genome size. The lengths of 18 chromosomes of the GT1 genome ranged from 36 Mbp (Chr17) to 104 Mbp (Chr 09) with an average size of 80 Mbp. Evaluating the quality of the assembled rubber tree genome, their results showed that more than 98% of NGS reads could be unambiguously represented with a high confidence of genome scaffolding. They aligned 51,701 expressed sequence tags retrieved from public databases and 102,235 unigenes assembled through RNA sequencing (RNA-seq) data of six GT1 tissues that covered 97.24% and 97.14% of protein-coding genes, respectively. Approximately 1042.42 Mbp (70.82%) of repetitive sequences were annotated, among which the GT1 genome comprised 65.88% (969.72 Mbp) of long terminal repeat (LTR) retrotransposons. They also predicted a total of 44,187 protein-coding genes with an overall support of 96.76% (of them, 79.34%, 75.80%, 94.70%,

and 96.37% from SwissProt, PFAM, TrEMBL and Interpro databases, respectively. Further, non-coding RNA (ncRNA) genes were annotated, yielding 945 transfer RNA (tRNA) genes, 93 ribosomal RNA (rRNA) genes, 61 small nucleolar RNA (snoRNA) genes, 396 small nuclear RNA (snRNA) genes and 373 microRNA (miRNA) genes. In addition, they annotated 689,255 SSRs, which is claimed to provide valuable genetic markers to assist future genetic improvement of the rubber tree. All the above-mentioned genomes reveal insights into different protein families in signal transduction, rubber biosynthesis, stress tolerance and plant performance that can be utilized for genomic designing, molecular breeding and developing commercially valuable rubber clones. Details of published whole genome assemblies of rubber is provide in Table 8.2. There is no doubt that data from these efforts will accelerate applied research on whole genome association studies, identification of potential markers, studying marker-trait relationship at whole genome level, genetic modification of rubberwith traits of interest along with high yield and improvement of germplasm.

8.5.3 Hevea *Transcriptomics*

Several research groups around the world have been working for the past two decade on various transcriptomes of *Hevea* to understand its biology, latex producing capability, disease tolerance, and climate resilience so that novel crop improvement strategies could be designed based on the information generated. One of the earlier reports on transcriptome was obtained from genes expressed in the latex of RRIM 600 by constructing cDNA library using regular/ subtractive hybridization (Han et al. 2000). Sequence analyses identified 245 ESTs, of which 57% showed homology to previously described sequences in public databases. About 16% of the database-matched ESTs encoding rubber biosynthesis-related proteins such as rubber elongation factor (REF) and small rubber particle protein (SRPP).

Recent high-throughput sequencing technology and various genomic tools have facilitated the data acquisitions in several crops in general and rubber in particular over the last two decades (Table 8.3). High-throughput genomic techniques are associated with innovative bioinformatics tools that assume much importance in breeding and can facilitate development of superior genotypes suitable for different agroclimatic conditions. Recent technological developments made RNA sequencing a cost effective tool in gene expression profiling while providing qualitative and quantitative information at whole genome level compared to other conventional methods (Xia et al. 2011). From clone Reyan7-33-97, more than 12 million reads with an average length of 90 nucleotides was generated. In total 48,768 unigenes (mean size = 436 bp, median size = 328 bp) were assembled through de novo transcriptome assembly (Xia et al. 2011). The assembled sequences have been annotated with gene descriptions, gene ontology (GO) and clusters of orthologous group (COG) terms. In total, 37,432 unigenes were successfully annotated. Mantello et al. (2014) reported transcriptome analysis from the bark of GT1 and PR255 clones and a total of 50,384

Table 8.2 A comparison of five published draft genomes of *Hevea* published

Genome details	Rahman et al. (2013)	Tang et al. (2016)	Lau et al. (2016)	Pootakham et al. (2017)	Liu et al. (2020)
Genotype	RRIM 600	Reyan 7–37-97	RRIM 600	BPM 24	GT1
Genome size	1.1 Gb	1.46 Gb	1.55 Gb	1.26 Gb	1.47 Gb
Scaffold number	6,08, 017	7453	1,89,316	5,92,580	600
Scaffold length	1,119 Mb	1.37 Gb	–	–	
Scaffold N50	2972 bp	1.28 Mb	67.24 kb	96.8 kb	87 Mb
Longest scaffold	531.5 kb	6.41 Mb	871.19 kb	2020 kb	
Contigs number	12, 23, 364	84,285	2,62,709	–	–
Total size of contigs	–	1.29 Gb	1.55 Gb	1.25 Gb	
Contigs N50	–	30.6 kb	20.75 kb	96.8 kb	152.7 kb
Longest contigs	–	312.7 kb	325.50 kb	2,026,921	
No. of protein coding genes	68,955	43,792	84,443	43,868	44,187
Mean gene length	1332 bp	3913 bp		2747 bp	3918 bp
Average coding sequence length	696	1123 bp	971 bp		
Average number of exons per gene	–	–	4.97	4	5.13
Average exon length	238 bp	308 bp	196 bp	223 bp	222 bp
Exon GC content		41.54		43.1	
Average intron length	332	677 bp	478 bp		
intron GC content		32.61		32.3	
Repeat sequence	71%	72%	72.5%	69.2%	70.81

Table 8.3 Details of NGS based transcriptome studies in *Hevea*

Publications	Sequencing method	Transcriptome type
Xia et al. (2011)	PE-RNA-Seq (Illumina)	Latex and leaf combined; clone RY7-33–97
Pootakham et al. (2011)	454 Pyrosequencing (Roche)	Leaf tissue; clone RRIM 600 RRII 105
Triwitayakorn et al. (2011)	454 Pyrosequencing (Roche)	Shoot apical meristem; clone RRIM 600
Chow et al. (2012)	RNA-Seq (Illumina)	Latex; clone RRIM 600
Li et al. (2013)	PE-RNA-Seq (Illumina)	Bark; clone RY7-33-97
Duan et al. (2013)	454 Pyrosequencing (Roche)	Leaf, bark, latex, root, embryogenic tissues; clone PB 260
Rahman et al. (2013)	PE-RNAseq (Illumina) 454 Pyrosequencing (Roche)	Leaf; clone RRIM 600 Leaf; clone RRIM 600
Salgado et al. (2014)	454 Pyrosequencing (Roche)	Pooled RNA extracts of different tissues and open pollinated seedlings; clone RRIM 600
Mantello et al. (2014)	RNA-Seq (Illumina)	Bark samples; clones GT1 and PR255
Zhiyi et al. (2015)	RNA-Seq (Illumina)	Latex; clone Reyan 7–33–97
Liu et al. (2015)	RNA-Seq (Illumina)	Bark; clone PR107

(continued)

contigs were obtained. A similarity search against the non-redundant (nr) protein database returned 32,018 (63%) positive BLASTx hits. The transcriptome analysis was annotated using the clusters of orthologous groups (COG), gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Pfam databases. Another group from Brazil reported transcriptome assembly from different tissues in RRIM 600 (Salgado et al. 2014), where a total of 17,166 contigs were successfully annotated. In this regard, it is also important to compare transcriptome data between clones, which was first reported by Li et al. (2015). They were able to find differentially expressed genes related to rubber biosynthesis pathway in RRIM 600 and RY 7-20-59 clones. Such studies can explain differences in latex yield potential of clones.

8.5.3.1 Transcripts and Non-coding RNAs Associated with Abiotic Stresses

Drought and low temperature stresses negatively influence plant growth and development by hindering several metabolic processes including stomatal conductance, nutrient uptake and photosynthetic assimilation that eventually reflects upon yield

Table 8.3 (continued)

Publications	Sequencing method	Transcriptome type
Chao et al. (2015)	Illumina paired-end sequencing	Latex; clone CATAS8-79 and PR107
Liu et al. (2016)	Illumina deep-sequencing	Bark; clone PR107
Li et al. (2016a)	RNA-Seq (Illumina)	Latex; clones CATAS7-33–97 and HAIKEN 2
Li et al. (2016b)	Illumina HiSeq 2000	Bark; Clone RY 7–33–97
Lau et al. (2016)	Illumina Hiseq 2500	Leaf, bark, latex; clone RRIM 600
Tang et al. (2016)	IlluminaGA2 and Hiseq2000	Different tissues and organs; clone Reyan7-33–97
Pootakham et al. (2017)	454 GS FLX + and Illumina HiSeq 2000 and PacBio RSII	Genome and transcriptome analysis
Sathik et al. (2018)	RNA-Seq (Illumina)	Drought and cold responsive transcripts from leaves of clone RRIM 600 and RRII 105
Montoro et al. (2018)	RNA-Seq (Illumina)	Ethylene and TPD responsive transcriptome from clone PB 260
Gong et al. (2018)	RNA-Seq (Illumina)	Cold stress tolerant transcriptome from Reyan 7–33-97
Zhang et al. (2019)	RNA-Seq (Illumina) and degradome analysis	laticifers of TPD trees
Roy et al. (2019)	RNA-Seq (Illumina)	Defense/Corynespora responsive genes

in rubber (Buttery and Boatman 1976; Sethuraj et al. 1984; Shinozaki et al. 2003; Sreelatha et al. 2007, 2011). To mitigate the adverse effect of stress, rubber plants often respond by altering at physiological, biochemical and gene expression levels. As a result of abiotic stresses, plants respond to varying environmental factors by triggering expression of multiple genes to mitigate the effect of stress (Zandalinas et al. 2020). Developing stress resilient crops is achieved by identification of key genes associated with such traits and signal transduction pathways and by selecting the newly bred varieties that harbor those specific traits without compromising on yield (Bailey-Serres et al. 2019). Studies on cold stress in rubber indicated significant increase in expression of cold responsive genes like carbonic anhydrase, glutathione peroxidase, metallothionein, chloroplastic Cu/Zn SOD, serine/threonine protein kinase, transcription factor, DNA-binding protein, etc. (Saha et al. 2010), LEA 5 protein, NAC tf and peroxidase (Sathik et al. 2012), CRT/DRE Binding Factor 1 (*HbCBF1*) (Cheng et al. 2015) and ethylene responsive transcription factor (ERF) (Sathik et al. 2018). Differential gene expression analysis in several rubber clones with varying levels of drought tolerance indicated the association of MAP Kinase, Myb tf, CRT/DRE binding factor and NFYA (Luke et al. 2015, 2017). Expression of

ferritin, DNA-binding protein, NAC tf and aquaporin were also found associated with drought tolerance (Sathik et al. 2018). Reactive oxygen species scavenging systems viz. *HbCuZnSOD, HbMnSOD, HbAPX, HbCAT, HbCOA, HbATP* and *HbACAT* were also found associated with drought stress tolerance (Wang 2014).

Higher level expression of *ErbB-3 binding protein 1* (*EBP1*) has been identified to enhance resistance to freezing and drought stress in rubber (Cheng et al. 2016a, b). Similarly four glucose-6-phosphate dehydrogenase (*HbG6PDH*s) gene families were reported to be involved in redox balance maintenance and defence against oxidative stress (Long et al. 2016). Stress studies by Gong et al. (2018) revealed that flavonoid biosynthesis, phenylpropanoid biosynthesis, plant hormone signal transduction, cutin, suberine and wax biosynthesis, pentose and glucuronate interconversions, phenylalanine metabolism and starch and sucrose metabolism were the significantly expressed main KEGG pathway genes. Furthermore, several hundreds of cold responsive transcription factors (TFs) viz., ARR-B, B3, BES1, bHLH, C2H, CO-like, Dof, ERF, G2-like, GRAS, GRF, HD-ZIP, HSF, LBD, MIKC-MADS, M-type MADS, MYB, MYB-related, NAC, RAV, SRS, TALE, TCP, Trihelix, WOX, WRKY, YABBY and ZF-HD were also found highly responsive to cold stress.

In 2020, Ma et al. identified 17 aluminium-activated malate transporters (ALMT) HbALMT from rubber trees and quantitative RTqPCR analysis revealed HbALMTs showed various expression patterns in different tissues. Transcriptome analysis and RT-qPCR assay showed that most of the HbALMT genes responded to aluminium stress, and among the 17, HbALMTs, HbALMT1, HbALMT2, HbALMT13, and HbALMT15 displayed higher expression levels in roots after two or five days of Al treatments, indicating their potential involvement in aluminium detoxification. In an experiment to study the effect of salinity on in vitro plantlets, 16 out of 20 genes showed a reduction in transcription after treatment with 300 mMNaCl. Only *HbmiRn12* was highly induced (4 to 6 times) in most tissues. A negative co-regulation between *HbMIR398b* with its chloroplastic HbCuZnSOD target messenger is observed in response to salinity (Gébelin et al. 2013a, b).

Transcriptome analysis identified that enhanced antioxidant protection in response to exogenous application of melatonin via regulating the genes involved in photosynthesis, ROS metabolism, flavonoids and melatonin biosynthesis. These results also indicated that melatonin can enhance salt stress tolerance directly or indirectly by counteracting H_2O_2 accumulation in rubber tree (Yang et al. 2019). In plants, gene expression is regulated by noncoding small RNAs (Storz 2002) like microRNA (miRNA), small interfering RNA (siRNA), small nucleolar RNA (snoRNA). Small rDNA-derived RNA (srRNA), small nuclear RNA (U-RNA) tRNA-derived small RNA (tsRNA) plays a major role in the various developmental stages of the plants and in regulating various biotic and abiotic stress responsive pathways. For example, miRNAs play main role in negatively regulating gene expression by binding with the complementary mRNAs to either cleave or repress translation (Chinnusamy et al. 2007). Regulatory role of miRNAs in rubber was first reported by Zeng et al. (2010) followed by Gébelin et al. (2012, 2013a, b) and Lertpanyasampatha et al. (2012). miRNAs that regulate MYB transcription factor, auxin responsive factor (ARF) and type III HD-Zip transcription factors were reported to be abundantly expressed in

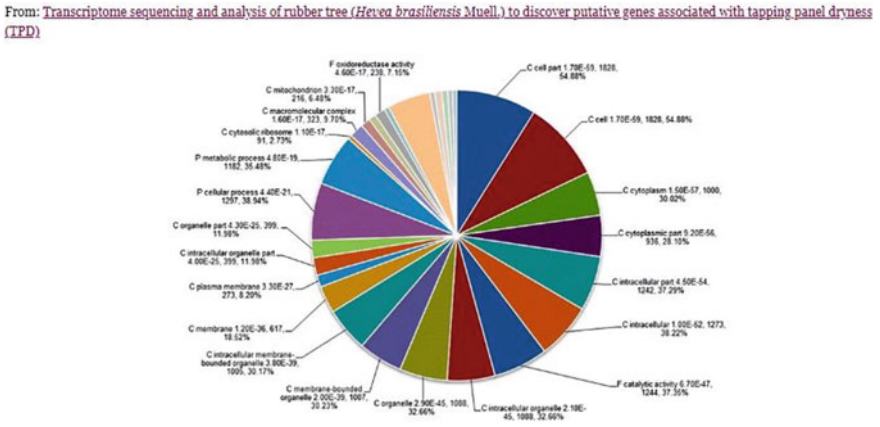

Fig. 8.2 Gene ontology annotation of down-regulated genes in the TPD-affected (T) bark compared to the healthy (H) bark of rubber tree. P: the biological process; C: the cellular component; F: the molecular function. The P value, the number of genes and the percentage of genes in the corresponding categories were given after the name of subcategories (Liu et al. 2015)

leaves. For example the miRNA MIR159b was found enhanced in leaves and bark in response to cold stress (Gébelin et al. 2013a). Enhanced expression of eMIR159b gene was observed in TPD affected trees also (Gebelin et al. 2013b). A number of genes which are up-regulated and down-regulated were identified in relation to TPD in rubber (Figs. 8.2 and 8.3) (Liu et al. 2015). Drought tolerance (miR482, miR164, miR167 and HbmiRn_42) and cold tolerance (miR169, miR159 and miR482) associated miRNAs were reported by Kuruvilla et al. (2016, 2017). Under cold stress, miR169 got down-regulated while its corresponding target mRNA (NF-YA) associated with stress tolerance got up-regulated (Luke et al. 2015). A novel miRNA (HbmiRn_42) that targeted HMG-CoA reductase was found highly up-regulated under drought conditions in drought tolerant clones indicating suppression of rubber biosynthesis under drought (Kuruvilla et al. 2016). All these studies indicate the possibility of using miRNA as markers for stress tolerance and in crop improvement programs in rubber (Sathik and Kuruvilla 2017). Overall, transcriptome studies have provided information on genes, their regulatory networks, differential response of transcripts to stress, candidate gene sequence information for the development of molecular markers for genetic linkage mapping, quantitative trait loci identification, investigation of linkage disequilibrium, etc.

8.5.4 Hevea *Proteomics*

Most protein data comes from translating gene sequences reported in public databases. However, some attempts were made to isolate proteomes of latex cells. Using 2D-gel electrophoresis and MALDI-TOF techniques 22 gene products related

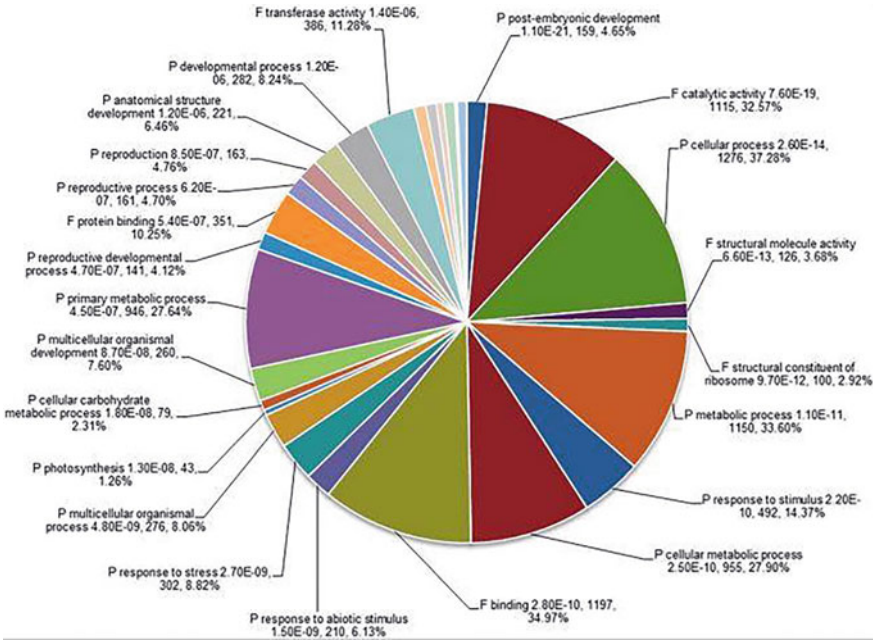

Fig. 8.3 Gene ontology annotation of up-regulated genes in the TPD-affected (T) bark compared to the healthy (H) bark of rubber tree. P: the biological process; C: the cellular component; F: the molecular function. The P value, the number of genes and the percentage of genes in the corresponding categories were given after the name of subcategories (Liu et al. 2015)

to rubber biosynthesis was identified from clone RY 7-33-97 (Xiang et al. 2012). From the same clone, proteomics of latex was analyzed using shotgun tandem mass spectrometry (Dai et al. 2013). A total of 186 rubber particle proteins were identified, with a range in relative molecular mass of 3.9–194.2 kDa and with isoelectric point values of 4.0–11.2. The rubber particle proteins were analyzed for gene ontology and could be categorized into eight major groups according to their functions: including rubber biosynthesis, stress- or defence-related responses, protein processing and folding, signal transduction and cellular transport. Habib et al. (2016) reports proteome from latex of RRIM 600. Proteins derived from rubber tree are the major contributors for allergy to natural rubber latex. When exposed to latex based products Type I latex hypersensitivity is observed in some occupational and other high-risk groups (health care workers, workers in the latex industry, children with spina bifida, and atopic individuals). Alenius et al. (1995) observed that prohevein, a latex protein was responsible for the induction of high frequency of IgE antibodies which suggests that this protein is a major natural rubber latex allergen. By proteomic approach, Yeang et al. (2002) identified latex proteins Hev b 9 and Hev b 10. Following these reports, several proteins associated with latex allergy were reported which lead to the development of allergen free latex based technologies and products.

Another area which substantially benefitted from proteomic studies is the rubber biosynthesis process. It was reported that geranylgeranyl pyrophosphate or cis-allylic pyrophosphate, geranyl pyrophosphate, dimethylallyl pyrophosphate and farnesyl pyrophosphate (FPP), could act as initiators of the in vitro oligomeric allylic pyrophosphate reaction during the chain elongation process of rubber biosynthesis mechanism (Cornish 2001a, b; Chiang et al. 2011). Another set of important proteins required for rubber biosynthesis especially the chain elongation process are the small rubber particle protein (SRPP) and the rubber elongation factor (REF) (Dennis and Light 1989; Oh et al. 1999; Kim et al. 2004). Genome sequencing studies and SDS PAGE-based proteomic analyses indicated that there are more than 10 homologous proteins in the REF/SRPP family (Rahman et al. 2013; Lau et al. 2016; Tang et al. 2016; Dai et al. 2013, 2017). Gene expression and protein estimation studies indicate that some members of this family are expressed more in latex than other tissues (Tang et al. 2016; Dai et al. 2016; Chow et al. 2007). Cisprenyl transferases (CPTs) are a set of prenyltransferases widely present in all higher organisms. However their role in rubber biosynthesis in rubber producing plants was discovered only very recently. In rubber producing plants they are primarily localized on the endoplastic reticulum and rubber particles (Qu et al.2015; Brown et al. 2017). Enzyme activity based assays have shown that they play a major role in determining the molecular weight of natural rubber. Functional characterization of CPTs revealed that the isoform HRT2 is the only isoform capable of catalyzing the synthesis of rubber molecule in the size range of 2×10^3–10^4 da (Asawatreratanakul et al. 2003). By reconstituting the rubber biosynthetic machinery in vitro, new components of the rubber transferase or prenyltransferase complex were identified by Yamashita et al. (2016). It is interesting to note that latex yield is determined not only by rubber biosynthesis but also by the flow of latex. Latex coagulation is a known player in determining the duration and rate of latex flow in rubber. Though numerous proteins were detected in lutoids, β-1,3-glucanase, hevamine, prohevein and hevein were identified as the most abundant among them all (Gidrol et al. 1994; Shi et al. 2010). Chrestin et al. (1997) show that the N-acetyl- D-glucosamine group is removed from the hevein receptor by a basic chitinase. All these studies indicate the major role played by proteins in determining the latex biosynthesis capability, latex allergenecity, latex flow, stress response, etc. and therefore a holistic approach combining genomics, transcriptomics and proteomics is the need of the hour to solve the mysteries behind the behavior of this wonder tree species.

The spurge in molecular techniques and information generated from molecular investigations in rubber has opened the avenue of rapid progress in crop improvement aspects. Further, incorporating the proteomic and metabolomics data can provide comprehensive picture in every respect. Markers can be best utilized to develop early selection methods for accurate prediction of mature phenotype at juvenile stage itself which is one of the main objective of rubber tree breeding. Incorporating genomics into breeding programs to identify high yielding genotypes would minimize the requirement of both space and time. It is anticipated that these genomic resources and tools generated may facilitate marker-assisted selection so that climate resilient smart

rubber clones with high latex yield and better disease tolerance cold be developed at the earliest for increasing NR productivity worldwide.

8.6 *Hevea* Epigenetic Research

Epigenetics is the study of modifications of the DNA or genes that are influenced by the environment which ultimately affect the way genes function. Unlike genetic changes, epigenetic changes are reversible in nature and are not directly involved in any changes in the DNA sequence as such. Instead they are simply modification of the DNA which may lead to DNA sequence independent phenotypic changes. Among the various epigenetic factors which modify or influence the way DNA is read, DNA methylation is the most common and extensively studied epigenetic factor. DNA methylation is a chemical modification of the DNA molecule where the normal cytosine in higher eukaryotes is modified to 5-methylcytosine (m5C) nucleotides. This epigenetic modification is very commonly observed in higher plants and animals. Studies pertaining to DNA methylation in plants suggest that they play an important role in regulating almost all major developmental processes of the organism concerned (Feng et al. 2010). Under biotic and abiotic stress conditions, plants undergo DNA methylation to alter gene expression related to numerous biochemical pathways involved in stress acclimatization and molecular adaptation (Finnegan et al. 1998). Hypomethylation in genome induced by cold stress (Chinnusamy et al. 2008) and drought stress (Labra et al. 2002) have proven that it is a well-synchronized strategy of the plants to alter gene expression to cope up with the changing environment. In rubber, abiotic stress influenced DNA methylation was reported in the promoter region of rubber biosynthesis genes and disease resistance gene (Uthup et al. 2011). Furthermore, the study also indicated the presences of genotype specific DNA methylation changes which have far reaching implication in the crop improvement programs of rubber. Tang et al. (2018) studied the chilling-induced DNA demethylation associated with cold tolerance by subjecting the plants to cold treatment followed by qRT PCR studies and bisulfite sequencing of cold-related genes such as HbICE1 and HbCBF2, cold-responsive (COR) genes, and DNA-methylation related genes such as HbMET1. They reported that DNA demethylation induced by cold was highly correlated with low temperature, but contrary to the earlier reports, no correlation was observed between de-methylation and the genetic backgrounds of cultivars in their study.

DNA methylation variations have been reported as the main cause of somaclonal variation in different crops (Gonzalez et al. 2013). By analyzing the epigenome, Rekha et al. (2015) showed that rubber plants developed by embryo culture was epigenetically more stable than plants derived by somatic embryogenesis or bud grafted plants. Using the same set of embryo culture derived plants, Uthup et al. (2018) proved that the epigenetic stability or uniformity of the plants generated from same embryo is lost after grafting procedure. This finding assumes great significance in rubber research as grafting induced epigenetic changes may be one very important

factor responsible for the high intra-clonal variation in terms of traits of interest seen in rubber cultivars despite being under same agroclimatic conditions. The above studies highlight the importance of epigenetic studies in rubber so that epialleles associated with stress may be identified which can complement genetic markers in identifying and developing environmentally stable climate resilient smart clones in the future.

8.7 Genoinformatics and *Hevea* Genome Databases

Research in natural sciences is multidisciplinary and requires less effort to extract a plethora of information. This huge information is interpreted into meaningful data using various analysis tools. For biologists, information from life forms is coded in the genetic material DNA or RNA and functions of these genetic code is carried out through proteins. With the advent of NGS techniques, it has now become a norm to obtain gigabyte-sized data sets of DNA or protein sequences which provide information of immense value to scientists in different fields such as population genetics, microbial ecology, plant breeding, drug development, molecular systematics and many others. This is where bioinformatics comes into play. Information obtained from genome or transcriptome is very valuable in plant breeding or genomic designing of a new variety. Looking at the perspective of improving phenotype of rubber; genes/proteins related to latex yield or rubber synthesis, tolerance to disease-causing agents or tolerance to cold and drought can be used in molecular breeding. This will drastically reduce the time and effort required to individually isolate each gene, study their function and then validate them.

With the generation of more and more data from rubber tree genomes, it is imperative to organize all the raw data deposited in public databases unified under a single database dedicated to rubber. A genome database thus acts a data bank which offers several options to select from and pick out the most desirable gene that have the potential to impart desirable phenotypic features. The HeveaDB 1.0 (http://hevea.catas.cn) mainly stores four versions of *Hevea* draft genome, 99 NGS transcriptomes, 18,451annotated EST sequences, 12 curated gene families, 30,200 gene annotations, 5,049 wild germplasm phenotype data, and 18,328 Wickham rubber clonal information. The HeveaDB provide user-friendly interface to facilitate scientists to utilize the data. Several tools are integrated in the database, including genome browser, blast, blat, text search, sequence retrieve and download (Cheng et al. 2019). The database has five major functional modules: Data, Search, Tools, Download and Submit. The complied gene page has information of each gene including ID, loci, structure, expression heatmap, co-expression network, sequences (genomic sequence, transcripts, CDS and deduced peptide) and gene annotations. The reference genome used in HeveaDB is from CATAS7-33-97 clone, which is visualized in the Gbrowse and Jbrowse respectively. Each gene is linked to its detail information page that can be accessed by clicking the gene names in the browser and the primary data and attributes of a gene are displayed, including name, position, length, and region

sequences and their sub-features. It is presumed that databases like HeveaDB will assist researchers in the rubber community in extracting valuable information to be utilized for the improvement and conservation of this tree crop.

8.8 Transgenics for Abiotic Stress

Genetic engineering or transgenic technology is more or less similar to conventional breeding in terms of the purpose of developing advanced crop varieties with new useful gene combinations. However it expands the possibilities by enabling introduction of useful genes not just from within the crop species or from closely related plants, but from a wide range of other organisms. The first genetically modified crop that was commercialized was the Flavr Savr tomato with improved shelf-life. Even though people were sceptical about genetically modified (GM) crops and its safety aspects, in the last 20 years, worldwide production of GM crops has been increased sharply. Over the past 30 years, the ability to alter life forms has been revolutionized by modern biotechnology.

8.8.1 Developing GM Rubber Plants for Abiotic Stress Tolerance

Stress is defined as an environmental factor contrarily affecting growth and development of the plant resulting in reduced crop yield. Major abiotic stresses include drought, high and low temperature and salinity which eventually reduce the crop productivity. Global warming and unpredictable climatic conditions due to the uncertainties in the rainfall pattern are causing rapid changes in the atmosphere. The reactive oxygen species (ROS) generated by drought stress in the chloroplasts and mitochondria (Apel and Hirt 2004; Asada 2006) are scavenged by enzyme systems mainly superoxide dismutase, peroxidase, catalase, glutathione reductase etc. and play a crucial role in drought tolerance responses. Drought stress mainly leads to growth retardation, shortening of the tapping period, blocking latex flow and TPD (Huang and Pan 1992).

Superoxide dismutase (SOD) is the first enzyme involved in the dismutation of superoxide radicals to O_2 and H_2O_2 and this enzyme activity was first reported by d'Auzac et al. (1989). This enzyme is encoded by a multigene family consisting of MnSOD of mitochondrial origin and two Cu/Zn SOD which are cytosolic in nature and another chloroplastic form (Miao and Gaynor 1993; Leclercq et al. 2012; Gébelin et al. 2013a). Higher levels of MnSOD were induced by ethephon treatment helping in preventing lutoid disruption by the superoxide radicals. But plants which are over exploited by ethephon exhibited higher free radical levels and lower SOD activity than the control plants (Das et al. 1998). Advancements in the molecular biology

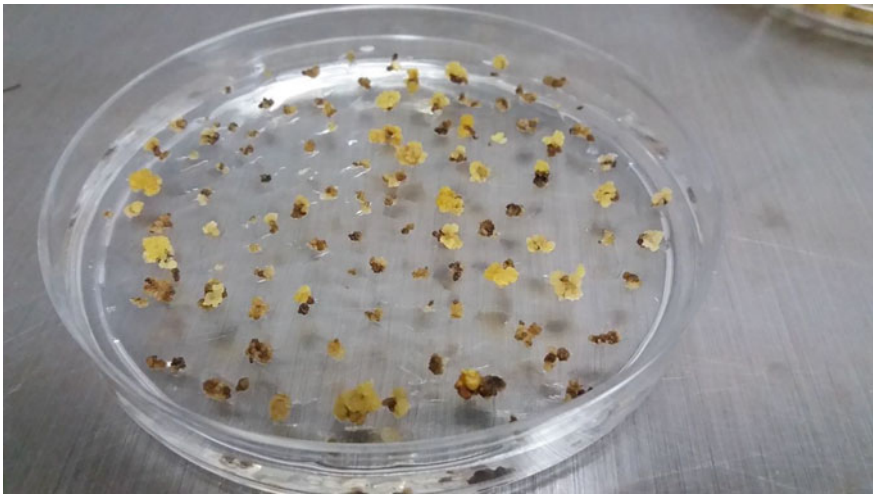

Fig. 8.4 Emergence of transgenic cell lines integrated with MnSOD

techniques in rubber provided newer insights into the functional genes related to drought tolerance. Representative genes in the mitochondria like *Hb*APX, *Hb*ATP, *Hb*MnSOD and *Hb*CuZnSOD were also cloned and characterized.

The incorporation of additional copies of SOD for enhancing stress tolerance was attempted for the first time at the Rubber Research Institute of India, by transforming immature anther derived primary callus with *Hb*MnSOD gene, which led to the development of transgenic rubber plants showing enhanced tolerance to abiotic stress (Jayashree et al. 2003; Sobha et al. 2003) (Figs. 8.4, 8.5 and 8.6). *Agrobacterium tumefaciens* strain EHA 101 carrying the binary vector pDU 96.2144 containing MnSOD gene under the control of *Cauliflower mosaic virus* (35S) promoter was used for infection. The drought tolerance capacity of the acclimatized MnSOD transgenic plants were evaluated and found that SOD enzyme activity was 35 and 31% higher under normal and drought imposed conditions in the experimented plants compared to control. Drought tolerance capacity of one year old MnSOD transgenic plants under water deficit conditions was evaluated (Sumesh et al. 2014). The experimental plants were maintained in the North Konkan region of Maharashtra and the physiological response of the plants was studied under stressed and re-watered conditions. The net photosynthetic (PN) rate declined in the control plants from the third day of water stress, while the reduction in the PN rate was lower in the transgenic plant (L1). Under re-watered condition, stomatal conductance and PN rate improved in transgenic plants while non-transgenic plants failed to recover after drought imposition. However, antioxidant enzymes such as SOD and peroxidase exhibited no definite trend in its activities (Sumesh et al. 2014). Further, Sobha et al. (2003) attempted *Agrobacterium*-mediated transformation with MnSOD gene under the control of *Figwort mosaic virus* 34S promoter. Leclercq et al. (2012) overexpressed a cytosolic *Hb*CuZnSOD gene in *Hevea* genome using embryogenic callus

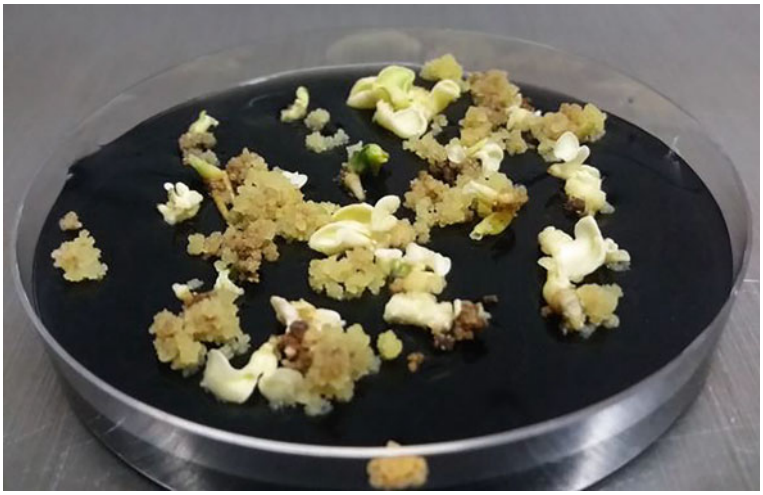

Fig. 8.5 Transgenic embryos integrated with MnSOD under maturation process

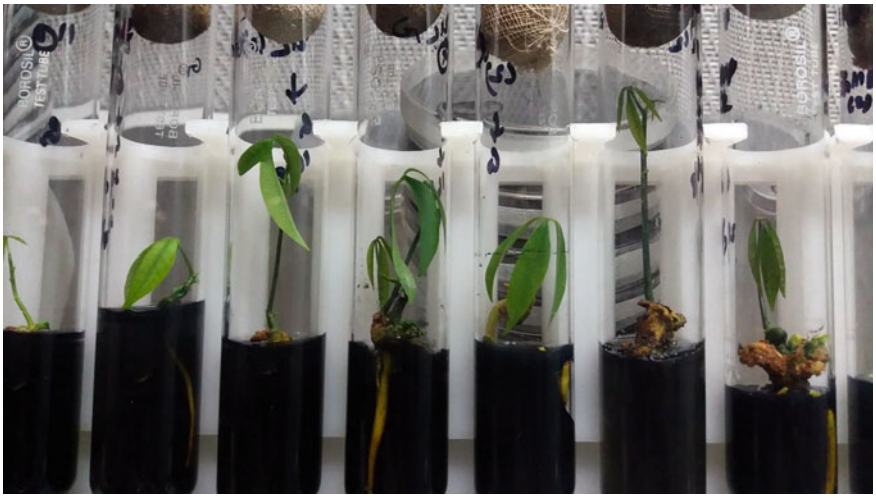

Fig. 8.6 Transgenic plants integrated with MnSOD

derived from integument tissue and could successfully develop transgenic plants. Drought stress tolerance of two overexpressing transgenic lines were evaluated in terms of physiological and biochemical parameters. After 21 days of dehydration, both transformed and untransformed control plants showed leaf symptoms associated with water stress, however, all transgenic plants remained healthy. Under water deficit condition, proline content was higher in transgenic plants compared to

control plants. Increased scavenging enzyme activity (SOD activity, ascorbate peroxidase and glutathione reductase) was also noticed in the transgenic plants. Moreover, transgenic plants exhibited a reduced stomatal conductance.

Osmotin gene, a multifunctional stress responsive protein from tobacco belonging to PR-5 family, was introduced into rubber for imparting abiotic/biotic stress tolerance (Rekha et al. 2013, 2014). Putatively transformed cells were subjected to drought and salinity stress with PEG and NaCl. An increase in the proline content was observed in the transformed calli subjected to PEG stress compared to the untransformed control callus. Plantlets were successfully acclimatized and established. Molecular analysis by PCR revealed the presence of *npt*II and osmotin genes in transformed cell lines. Transgene integration and expression in the putative transformants were confirmed by Southern blot and reverse transcription-PCR analyses (Rekha et al. 2014). Attempts were made to develop drought/cold tolerant plants by introducing an apple cDNA encoding sorbitol-6-phosphate dehydrogenase gene (S6PDH) into rubber (Jayasree et al. 2015). Transgenic embryos and a few germinating embryos were developed in vitro from the putatively transformed cell lines. Transgenic rubber plants tolerant to wind damage were developed by transforming rubber genome with *Arabidopsis* gibberellic acid insensitive gene (*GA*1) by microparticle bombardment. Stable gene integration in the leaves of transgenic plants was confirmed by PCR and Southern blot analyses (Wang et al. 2013). Abiotic stress tolerant transgenic plants with enhanced growth parameters were developed using ethylene responsive factor gene (*HbERF-1Xc5*). Plants overexpressing this gene accumulated more starch, thereby differentiated more laticifers as evidenced by histological studies (Lestari et al. 2018).

Tapping panel dryness (TPD) syndrome characterized by partial or complete stoppage of the latex is a physiological disorder resulting from the overexploitation of rubber tree (Faridah et al. 1996). ROS generation due to the high NADPH oxidase activity at the surface of the lutoids leads to peroxidative degradation of the lutoids resulting in bursting (Zhang et al. 2016). TPD related mRNA transcript levels were studied in rubber tree using suppression subtractive hybridization (SSH) method (Venkatachalam et al. 2007). Experimental results revealed a reduction in the expression of two genes namely Myb transcription factor and translationally controlled tumor protein (TCTP) gene in TPD affected trees compared to healthy ones. Later two forms of TCTP gene were cloned and characterized from rubber by the Chinese group (Li et al. 2013; Denget al. 2016). Overexpression of *Hb* TCTP gene was attempted in tobacco and enhanced tolerance to oxidative stress was observed (Deng et al. 2018). A reduction in the cytokinin levels in TPD affected trees specified the role of this growth regulator in TPD occurrence (Das et al. 1998). To address this issue, attempts were made to overproduce cytokinin in transgenic tissues using isopentenyltransferase (*ipt)* gene. However, embryos showing developmental abnormalities were obtained along with normal embryos. The severely deformed embryos were unable to regenerate whereas normal embryos were capable of regenerating transgenic plants (Kala et al. 2003, 2019). The attempts for genetic transformation in *Hevea* and the results are summarised in the Table 8.4.

Table 8.4 Summary of transgenic work for abiotic stress tolerance in *Hevea* worldwide

Plasmid vector	Gene	Transfer method	Target tissue	Result	References	Country
pDU 96.2144	MnSOD,GUS,	*Agrobacterium* Strain EHA 101	Anther callus	Transgenic plants	Jayashree et al. (2003)	India
pDU 96. 2412	*MnSOD GUS, npt*II	*Agrobacterium* Strain EHA 101	Anther callus	Transgenic plants	Sobha et al. (2003)	India
pDU96.2144 pDU.96.2111	*MnSOD*, *GUS, npt*II	*Agrobacterium* EHA101	Anther callus	Increased frequency of transformation	Rekha et al. (2006)	India
pCAMBIA2301 pCAMBIA2300	*uidA, CuZnSOD, GFP, npt*II	*Agrobacterium* EHA 105	Anther callus	Transgenic plants	Leclercq et al. 2012	France
pDU 97.0612	*IPT*,*GUS, npt*II	*Agrobacterium* EHA 101	Anther callus	Transgenic embryos, Transgenic plants	Kala et al. (2003, 2019)	India
pBin19	*Osmotin, npt*II	*Agrobacterium* GV2260	Anther& zygotic callus	Transgenic plants	Rekha et al. (2013, 2014)	India
pDU 93.03.05	*Sorbitol*, *GUS, nptII*	*Agrobacterium* EHA101	Anther callus	Transgenic embryos	Jayasree et al. (2015)	India
pBI121	*GAI*, *GUS*, *Npt*II	Particle bombardment	Anther callus	Transgenic callus	Wang et al. (2013)	China
pCamway	*HbERF-IXc5* with *CaMV*35S, *HEV2.1*promoters, *GFP*	*Agrobacterium* EHA105	Integument calli	Transgenic plants	Lestari et al. (2018)	France

8.9 Biosynthesis, Biotransformation, Metabolic Pathway Engineering and Gene Discovery

Isoprenoids represent an array of compounds having potential applications in the development of pharmaceutical, neutraceutical and quality chemicals products. NR is an important macromolecule having industrial applications. Plants use two pathways namely MVA (mevalonate) and MEP (methyl erythritol 4-phosphate) to synthesize IPP (Isopentanyl pyro phosphate) which is the monomer of NR. The intermediates derived from sugar metabolism serve as the substrate (pyruvate and glyceraldehyde 3-phosphate or acetyl-CoA) for MEP and MVA pathway respectively. Acetyl Co A from sucrose, glucose or fructose is the primary substrate for the cytosolic MVA pathway and is considered the well documented route for IPP formation. Initiation of the rubber chain requires an allylic pyrophosphate initiator like DMAPP, GPP, FPP, GGPP etc. For IPP incorporation, rubber transferase enzyme needs divalent cations Mg^{2+}/Mn^{2+} as cofactors. By the continuous incorporation of IPP monomer, rubber molecules grow using living carbocationic polymerization.

The importance of isoprenoids engendered research aimed at overproduction of these compounds by metabolic pathway engineering. Modifying the network of biochemical reactions using recombinant DNA technology leading to a specific goal is termed as metabolic engineering (Stephanopoulos and Sinskey 1993). The aim is to redirect carbon and energy fluxes to achieve a particular objective, especially to increase the rate of production and also by minimizing the formation of undesirable side products. Determining the flux by the ^{13}C labelled NMR studies had enormous impact on the growth of this important technology (Stephanopoulos and Sinskey 1993). For fulfilling these objectives, we have to find out the critical junctions in the matrix, specific enzymatic modifications required for bringing the final result and select an appropriate type of genetic modification needed for achieving it. Metabolic rigidity is considered as a determining factor of flux distribution at the branch points of the grid. First step towards metabolic engineering is to identify the branch point having direct bearing on product yield. After identifying the node, appropriate modification of the enzyme i.e., amplification, inhibition or deregulation may be chosen.

The use of genetic means for the redistribution of the metabolic flow can be replaced by use of bioreactors (Kiss and Stephanopoulos 1991). This approach mainly aims to move complete biosynthetic pathways from the native host to heterologous organisms to improve yield. Transfer of this heterologous metabolic pathway from a native host to a standy or proxy microbial host is also challenging since the pathways may always be multi step requiring many cofactors or energy barriers like NADPH, ATP etc. to be active (Martin et al. 2003; Yan et al. 2005; DeJong et al. 2006). Methods like DNA assembler technique enabled the discovery, characterization and engineering of natural products by designing and constructing metabolic pathways in one step (Shao et al. 2012). During optimization of the targeted product synthesis two approaches were examined. First one is process analysis which optimizes the operational conditions such as temperature, pH, nutrients, aeration etc. Biocatalyst

analysis is the second parameter that organizes and reconstructs the data related to intracellular mechanism. Still the expression of the pathway as a whole into a host organism for the production of natural products is very difficult as this reconstruction involves many permutations and combinations. But as this strategy has high economic potential, perspectives of such a pathway engineering and impacts may be considered. Later advances in metabolic engineering and synthetic biology set forth the use of recombinant expression systems to reestablish/reconstruct natural product pathways.

A method for the de novo formation of rubber particle was elaborated by Archer and Audley (1987) where the molecular weight and uniqueness of the rubber formed in vitro was discussed. Rubber latex serum was incubated with ^{14}C labeled isopentenyl pyrophosphate and all *trans* ^{14}C geranyl geraniol were isolated. Authors explained the biosynthesis of rubber, a poly isoprene originating on the surface of existing small particle. According to them, IPP was isomerized to DMAPP which is complexed with lipoprotein and this primary isoprene unit developed to the C_{20} stage (geranyl geranyl pyrophosphate) partly in the *trans* form. Isoprene units from IPP were then added on to form high molecular weight rubber. Later the structure of rubber formed in vitro was described by Tangpakde et al. (1997) using fresh bottom fraction. Differences in the rubber initiating molecules both in vitro *a*nd in vivo was analyzed and the study indicated FDP as a direct initiator for the rubber produced in vitro. The rubber formed in vivo utilized an unidentified initiating molecule having two trans isoprene units. Merkulov et al. (2000) attempted biosynthesis of c*is* 1,4 polyisoprene in oleaginous yeast, *Yarrowia lipolytica* by silencing the squalene synthase gene of yeast. Paper discussed the silencing effect of squalene synthase gene on activation of rubber transferase expression with precursors for polymerization.

Generally, *E. coli* and *Saccharomyces cerevisiae* were used to avert technical issues related with metabolic engineering. Though *E. coli* has many advantages to be used as an ideal host including its comparatively simple metabolism and robust regulatory system, it is not considered as a preferable host. Reason is the difficulty in expressing complex enzymes, poor stress tolerance capacity and lack of mechanism for post translational modifications which favored yeast as an exemplary system to work with. Yeast system is an ideal one due to the low growth temperature, tolerance against pH, side products etc.

All isoprenoids are developed from the prenyl phosphate precursors IPP and DMAPP supplied through MEP or MVA pathway. As they share common metabolic precursors, a variety of valuable compounds can be synthesized by engineering these metabolic pathways. Many reports demonstrated a cross talk between these two spatially separated cytosolic and plastidic pathways. Kumar et al. (2012) used the metabolic engineering approach to express multiple genes of MVA pathway in the chloroplasts of *Nicotiana tobacum.* Regenerated transplastomic plants showed enhanced levels of mevalonate and cytoplasmically synthesized carotenoids, indicating shuttle of excess IPP from the plastids to the cytosol. However qRT-PCR studies proved that the compartmentalization of plastidic IPP for cis-polyisoprene formation is related to the degree of plastidic carotenoid synthesis (Sando et al. 2008). So MEP pathway may serve as an alternate contributor of IPP for rubber biosynthesis

in mature trees which do not produce large amount of carotenoids. Thus two specific routes existed in one species for IPP partitioning and utilization (Chow et al. 2012; Lau et al. 2016).

Isoprene production through metabolic engineering of microbial system is feasible and *E. coli* system has been widely employed for the bioproduction of isoprenes especially terpenoids (Klein-Marcuschamer et al. 2007; Martin et al. 2003). Terpenoids having pharmaceutical value including taxol, artemisinin and lycopene have been synthesized. Taxadiene, used in anticancer treatment was synthesized with good yield in a heterologous host utilizing MEP pathway (Boghigian et al. 2012). Farmer et al. (2000) attempted lycopene production in *E.coli* with *glnAp2* promoter serving as a control valve regulating gene expression according to the acetyl phosphate level. In a different attempt using a logical strain design, IPP production was rapidly improved by expressing the mevalonate pathway from *Saccharomyces cerevaesiae* and *Streptomyces* in *E. coli* (Martin et al. 2003; Vadali et al. 2005). Experiments were designed to enhance precursor levels of the enzymes between pyruvate and glyceraldehyde 3 phophate utilizing MEP pathway in *E. coli*. Use of multi copy vectors for increasing the product yield was suggested by researchers but the load posed by the vectors caused metabolic burden on wild type *E. coli* strain restraining its growth (Jones et al. 2000). The accumulation of one or more metabolic intermediates reduced the net yield of the final product and resulted in deceleration of microbial growth as they were unable to tolerate the increased titers of the pathway intermediates (Stephanopoulos 2002; Wei et al. 2001). Therefore balanced reaction fluxes are needed to increase the yield of the product. These studies pointed out the importance of post transcriptional processes like mRNA stability and translational initiation in modulating the fluxes (Smolke et al. 2001). Random mutagenesis of the house keeping sigma factor of *E. coli* resulted in 50% improvement of lycopene production in the modified strain (Alper and Stephanopoulos 2007). Precursor flux to the MVA pathway was considerably improved by overexpressing acetaldehyde dehydrogenase gene and acetyl-CoA synthetase genes in *S. cerevisiae* (Shiba et al. 2007). High titers of the final product were synthesized by selecting MVA pathway for developing robust strains (Immethun et al. 2013).

An attempt was carried out by Team Stanford to pacify the demand shortfall of NR by developing an alternative system stimulating the process of natural rubber production in a transgenic organism. This system utilized MEP pathway after identifying the rate limiting enzymes of the pathway. A plasmid with complete DXS synthase gene was created with ampicillin resistance. Regulated production of DXS was optimized by introducing the enzyme into *E. coli* under an IPTG inducible constitutive promoter. Thereby a greater output of the downstream enzymes like IPP and DMAPP was obtained. To enable the synthesis of isoprene polymers, a second component containing cis prenyl transferases (CPT) and SRPP genes needed for the extension was introduced. These fragments were linked via Gibson assembly to yield a cassette of genes. This construct referred as latex operon was subsequently used for transformation. Here the cells were supplemented with Magnesium, glucose and cofactors to augment the enzyme activity. Thus through metabolic engineering they were successful in developing an organism capable of producing rubber like

substance exhibiting basic properties of latex (Team iGEM 2016). In continuation with the above work, theoretical yield between the two pathways (MVA and MEP) were compared and proved that the non mevalonate pathway displayed the highest theoretical yield and therefore used this route for polyisoprene production (Acharya et al. 2018).

NR biosynthesis in plants naturally occur in a cellular environment and moreover the elongation of polyisoprene chain occurs on the surface of rubber particle. As we explore the possibility of rubber biosynthesis in microorganisms, the cellular concept will never work and the elongation of the polyisoprene occurs as insoluble inclusions rather than relying on presence of soluble enzymes. These insoluble inclusions deposited on the cytoplasm of the recombinant cells might create a problem to the host cell which may affect the stability of these strains if they are unable to degrade the compound. Other hydrophobic proteins bind irreversibly and nonspecifically to these hydrophobic surface provided by the rubber inclusions thereby increasing the surface area of such inclusions. Therefore co-expressions of specialized proteins to cover the surface of rubber inclusions are needed in recombinant microorganisms to stabilize the rubber developed in vitro (Kim et al. 2002). Eventhough isoprene production through cell based systems has many disadvantages which include nutrient limitations, accumulation of toxic intermediates hindering cell growth and limited yield which demands emergence of cell free systems employing synthetic biochemistry. Due to these complexities of the cellular metabolism, an alternative approach was used to build biochemical pathways in vitro, using synthetic biochemistry. Full mevalonate pathway was reconstituted in vitro to produce the commodity chemical isoprene (Korman et al. 2014). An in vitro synthetic biochemical pathway was constructed which uses the carbon and ATP from the glycolysis intermediate phosphoenol pyruvate to run the mevalonate pathway. By balancing the ATP, NADPH and acetyl CoA cofactors the complex transformation procedures were standardized using 12 enzymes. Thus glycolysis was utilized to power synthetic biochemical reactions and appropriate utilization of ATP resulted in the production of isoprenoid compounds at a higher rate. The study proved that by inserting isoprene pathway into the already developed glycolysis module, acetyl CoA derived isoprenoids can be synthesized from glucose in vitro. But Cheng et al. (2017) focused on synthesizing isoprene directly from MVA utilizing an enzymatic process consisting of lower MVA pathway enzymes. Four biosynthetic enzymes of lower MVA pathway and isoprene synthase enzyme (ISPS) were prepared individually in recombinant *E.coli* strains and purified before in vitro production. Optimum mevalonate required for maximum production of isoprene was standardized (2.5 mM) and isoprene production was increased with increasing amount of ATP from 2 to 12 mM under optimum substrate concentration. Thus the in vitro experiment identified two enzymes namely isopentenyl diphosphate isomerase (IDI) and isoprene synthase (ISPS) as essential in the pathway for isoprene production from mevalonate and balanced expression of these enzymes to be a key determinant in isoprenoid formation. An optimized molarity ratio of the lower mevalonate pathway enzymes serving as a key towards isoprenoid production was deducted from the study and the ratio of the respective enzymes MVK: PMK: MVD: IDI: ISPS was 1:1:1:2:16.

Termination of polymerization or the molecular weight determinant is the concentration of FPP/IPP used or their ratio as initiator molecule (Cornish et al. 2000; Cornish 2001a, b). Rubber quality is determined by the molecular weight and molecular weight is also altered by the concentration of metal ion cofactor/ activator (Scott et al. 2003; Bushman et al. 2006; Cornish and Blakeslee 2011). As the molecular weight is strongly correlated with the quality of the product, factors regulating molecular weight of rubber was investigated through in vitro studies (Cornish et al. 2000). Enzymatically active washed rubber particles prepared from fresh latex was utilized for the experiment. Mean molecular weight of rubber increased with increasing concentrations of IPP monomer when FPP concentration was limiting. A decreasing trend in the mean molecular weight was noticed with increasing FPP concentrations. Thus rubber polymer initiation, rate and molecular weight were dependent on substrate concentration and the ratio between IPP and FPP. The active site of the rubber particle was capable of accepting a wide range of allylic pyrophosphate initiators as established through in vitro studies. The rubber transferase showed higher affinity towards benzophenone modified geranyl pyrophosphate (Bz-GPP) and dimethylallyl pyrophosphate (Bz–DMAPP) analogues with ether-linkages (Xie et al. 2008). Understanding the genes involved in the final polymerization step of the pathway provide better insight for improving the crop.

NR is synthesized by the rubber transferase enzyme which is a membrane bound protein complex with several subunits. Cis prenyl transferases (CPT) is one component of the protein complex which catalyze the incorporation of IPP into allylic diphosphate co-substrate to initiate polymerization in presence of divalent cations Mg^{2+} or Mn^{2+} as co factors. Two CPTs were cloned from rubber latex, *Hevea* rubber transferase 1 and 2 (HRT1, HRT2). Both the enzymes have conserved regions for catalytic function and substrate binding sites for chain elongation enzymes. The rate of rubber biosynthesis is faster under in vitro conditions using longer APP molecules of C^{15} or C^{20} (Cornish and Siler 1995; Cornish et al. 1998; Archer and Audley 1987). Addition of HRT2 protein to washed bottom fraction prepared from centrifuged rubberlatex resulted in increased synthesis of new rubber molecule (Asawatreratanakul et al. 2003). But according to Yamashita et al. (2016), addition of HRT-1 (instead of exogenously added IPP or FPP) into partially deproteinized washed rubber particle regained RTase activity producing minimal amount of rubber. But the latex specific CPT requires eukaryotic cell specific factors along with some additional factors in the latex to exhibit RTase activity. Supportive evidences was given by Takahashi et al. (2012) where the expression of the recombinant proteins HRT1 and HRT2 failed to exhibit rubber transferase activity in *E. coli* while expression in the eukaryotic system supported the formation of polyisoprenoids of shorter chain length. Involvement of CPT on NR formation was once again proved in the knock down experiment using RNAi in the latex of *Taraxacum brevicorniculatum* (Post et al. 2012). But in vitro studies proved that rubber transferase is not the prime determinant of molecular weight. In addition to substrate availability; other endogenous factors may also be operating in vivo for regulating the molecular weight including some integral proteins and other membrane constituents like unusual furonoid fatty acids predominantly seen associated with rubber particles membrane (Siler et al. 1997).

Rubber biosynthesis occurs on the surface of the rubber particle which accounts for about 30–50% volume of latex. They are pear shaped sub cellular organelles made of polyisoprene surrounded by lipids, proteins and other compounds. Rubber elongation factor (REF) and small rubber particle proteins (SRPP) are important proteins having a role in rubber particle stabilization and coagulation. These enzymes are needed for NR synthesis since they promote incorporation of IPP into whole latex. A cell free translation coupled recombinant protein system was introduced on detergent washed rubber particle in order to identify the protein complex which functions as NR biosynthetic machinery on the rubber particle. Accurate introduction of CPT on washed rubber particle acted as a key for the expression of RTases and the formation of HRT-1 containing ternary complex is needed for efficient rubber production on rubber particle (Yamashita et al. 2016). Molecular mechanism of rubber production on rubber particle was identified through this study. In *vitro* translation coupled protein reconstitution on washed rubber particle (WRP) proved the expression of HRBP and REF along with HRT1 for efficient rubber biosynthesis in *Hevea.* Recently, Chatzivasileiou et al. (2018) illustrated the production of two main isoprenoid intermediates IPP and DMAPP from externally supplied isoprenol as the substrate. This enzymatic pathway utilized a single cofactor, ATP in two simple steps. Thus the isopentenol utilization pathway (IUP) as an alternative to isoprenoid pathway is a remarkable advancement in the field of isoprenoid biosynthesis.

8.10 Recent Concepts and Strategies Developed

8.10.1 Nanotechnology

Nanotechnology has been used in the field of agriculture to enhance the crop production. The use of engineered nanomaterial has revolutionized world agriculture. The reduction of environmental pollution and protection of the clean environment is the major challenge in sustainable agriculture, and nanomaterials bring forth an assurance of better management and conservation of inputs to plant production. The potential of nanomaterials offers a new green revolution with less farming risks. However, our knowledge about the behavior and fate of altered agriculture inputs and their interaction with bio-macromolecules, the uptake capacity, permissible limit and the ecotoxicity of different nanomaterials is still not sufficient enough to use them. Hence further research is urgently needed in this direction.

In rubber, use of nanoparticles for cultivation is not reported so far. However, a few reports on the use of the technology in rubber processing is available. Enhancement of mechanical properties of rubbers is carried out by the dispersion of nano-dimensional solid particles like carbon black and silica. Such fillers are known as reinforcing fillers which remarkably increases the abrasion resistance, hardness, resistance to crack growth etc., of the rubber compound after vulcanization. A highly successful example is the use of nano-sized carbon black to improve the wear resistance of tyres.

The particle size of the filler, uniform dispersion and rubber filler interaction play the significant role towards the reinforcement. Other fillers with at least one dimension in nano-range like layered silicates (clay) in unmodified and modified forms, carbon nanotubes (CNTs), graphene and layered double hydroxides are also used, though with limited commercial success to prepare reinforced rubber products (Das et al. 2014).

8.10.2 Gene Editing

CRISPR/Cas9 mediated genome editing is the latest and widely accepted technology for manipulation of genes. Theoretically, it is possible to knock in, knock out and regulate genes according to our needs. The system was developed by Jennifer Doudna and Emmanuelle Charpentier (2014) who received Nobel Prize in the year 2020. The technology of genome editing relies upon sequence specific nucleases (SSNs), which are capable of generating DNA double strand breaks at specific locations within the genome (Agneset al. 2017). As a result, the error prone natural endogenous DNA repairing mechanism will be triggered to repair the DNA damage which aids in targeted mutagenesis. Generation of stable and heritable mutants without disturbing the existing genetic composition and the ability to develop homozygous (w. r. to that particular loci) edited plants in one generation make the CRISPR technology more attractive. Research on CRISPR/Cas9 in model systems and other plants provided strong evidence for the use of this technology for developing improved varieties with genome modification and stable inheritance. In rubber also, there are immense possibilities of exploiting genome editing technologies like CRISPR/Cas 9 as it eliminates many of the regulatory burdens associated with field planting and commercialization of GM rubber clones developed through conventional plant transformation technologies. Editing of defined loci eliminates the possibility of interfering with the activity of other genes. Screening of large number of events can also be avoided as the editing is precise and site specific and regeneration of one edited event is enough to serve the purpose.

So far, very few reports are available for genome editing of rubber tree and other rubber yielding plants. In Russian Dandelion rubber *Taraxacum kok-saghyz*, knocking out the gene encoding fructan: fructan1-fructosyltransferase (*1-FFT*), involved in inulin biosynthesis was carried out successfully using CRISPR/Cas9 mediated genome editing. Inulin is supposed to be an antagonist of rubber production. The regenerated plants contained knockout alleles with high mutation rates (80.0%) (Laffaldano et al. 2016). Targeted mutagenesis in rubber tree was attempted for the first time in China using direct delivery of CRISPR/Cas9 ribonucleoproteins (RNPs) through gene gun using protoplasts as target tissue, for targeting two genes (*FT* and *TFL1*) genes and mutation frequencies ranging from 3.74–20.11% were reported (Fan et al. 2020). In rubber, CRISPR technology can be exploited for increasing yield by redirecting the metabolic flux of non-rubber pathways to natural

rubber biosynthesis, removal of allergens through knocking out of respective genes, elucidation of unknown pathways and improving abiotic and biotic stress.

Availability of *Hevea* whole genome sequence and well established transformation system make rubber tree an ideal candidate for gene editing. However the full benefit can be exploited if DNA free editing of single cell could be achieved. This ensures the absence of undesirable foreign genetic elements and thus the regulatory burdens associated with the conventional transgenic approach can be bypassed. However extensive research is warranted in this direction.

8.11 Brief Account on Social, Political and Regulatory Issues of GM Rubber

From the first transgenic plant developed during 1996, concerns have been expressed by environmentalists about the potential risks associated with GM plants, mainly on their impact to human health, environment and biological diversity. In genetically modified plants, other than the gene of interest, there are marker and reporter gens for the identification and selection of transformants. Usually the marker genes are meant for antibiotic resistance and this is one of the major concerns for propaganda against transgenics. The major biosafety concerns were risk of toxicity, due to new product developed through gene alteration, newer proteins which may be allergenic and use of antibiotic resistance as selectable marker genes with potential risk of aggravate the health problems due to antibiotic resistance. Gene flow due to cross pollination, erosion of biodiversity due to monoculture, gene escape into the environment and pollution of gene pools are other potential risk factors. Realizing the requirement of biosafety in genetic engineering research and development activities, Cartagena Protocol on Biosafety (CPB)" has been adopted by 167 parties on 11 September 2003. This is an international multilateral agreement on biosafety. According to this research work in the area of GE and GMOs requires prior approval from the appropriate regulatory authorities of the country. Following guidelines provided for minimizing biosafety issues is mandatory. In India, the primary regulatory body at research institute level is the Institutional Biosafety Committee (IBSC) which ensures safety measures. Biosafety regulations cover assessment of risks and the policies and procedures adopted to ensure environmentally safe applications of biotechnology. All the utensils, materials and tools used for transformation experiments need to be autoclaved. Transgenic plants developed are to be grown in containment facility. Unwanted plant materials such as dried leaves etc. should be removed and incinerated periodically. Before going for field planting, even for a mini field trial permission from Govt. of India is essential. The application for field trial is routed through Review Committee for Genetic Manipulation (RCGM). After clearance from RCGM, permission will be given by Genetic Engineering Appraisal Committee (GEAC). No person can establish a CFT of any GE plant in India without the prior approval of RCGM and GEAC. Also, a no objection certificate from the

State Govt., where the planting site is situated is essential. Appropriate isolation distance should be maintained in the field. Periodic data collection and studies on the change in micro-flora after planting GM if any should be studied. After ensuring the fact that no harmful effects are recorded in connection with planting of GM, and obtaining appropriate permission from the regulatory authorities, the commercial planting will be materialized. However, the health hazards to human beings and other living organisms are more relevant in the case of food crops. Since rubber is not an edible crop, food safety issues with rubber transgenic are irrelevant. Asynchrony of flowering among clones and wild relatives reduces the chance of gene flow. Centre of origin of rubber is the Amazon river basin in South America and rubber cultivation is predominant in South Asian countries where the availability sexually compatible wild relatives are rare and risk of gene flow is remote. However, while going for field planting, reproductive isolation, planting boarder trees and destruction of unwanted materials after the trials need to be strictly followed.

8.12 Future Perspectives

Since there is an ever increasing demand for NR, enhancement in productivity is the need of the hour. Uniformity in yield for the same clone in same plot is seldom achieved in rubber, due to several reasons. Variability of the rootstock is a major factor responsible for this molecular evidences are reported for the stock induced variations in the scion (Uthup et al. 2018). Identification and multiplication of elite root stocks is an under exploited area in rubber breeding. For example in drought prone areas, development of drought tolerant rootstocks may be a viable option.

A theoretical yield of 9000 kg/ha was predicted for rubber (Jacob et al. 2021). In rubber, the biomass production is always high and therefore, availability of carbon for biosynthesis of rubber is not a limiting factor. In this context, a combination of a high biomass producing genotype and physiologically active genotypes with higher rate of latex production is suggested for developing new varieties (Jacob et al. 2021). For identification of promising parents as well as their best progenies at an early stage, molecular tools can be effectively utilized. A fruitful combination of conventional breeding and modern biotechnology tools for deriving new varieties will accelerate the development of varieties with high productivity.

Latest molecular tools like gene editing can be employed for deriving unknown pathways for biosynthesis, redirecting non rubber pathways for rubber synthesis, achieving abiotic and biotic stress tolerance. Identification and manipulation of genes for abiotic stress tolerance and developing genetically modified plants suitable for different climatic zones can have a big impact in rubber production. With the release of whole genome sequences of rubber, the identification of genes and its manipulation can be done in a more precise way and a quantum leap in productivity can be expected.

References

Abraham J, Jessy MD (2018) Soil CO_2 flux measurements from a mature natural rubber plantation. Rubb Sci 31(3):196–207

Acharya N, Baker N, Bravo MK Katie Gu, HarkenS et al (2018) Getting there and staying there: supporting and enabling persistent human life on Mars using synthetic natural rubber, self-healing materials, and biological batteries. bioRxiv:345496

Agnes R, Paulin C, Wendy H (2017) Use of CRISPR system in plants genome editing toward new opportunities in agriculture. Emerg Top Life Sci 1:169–182

Alam B, Das G, Raj S, Roy S, Pal TK, Dey SK (1998) Biochemical determinants of seasonal and clonal yield performance of *Hevea brasiliensis* under agroclimatic conditions of Tripura. In: National symposium on future goals of physiological research for the improvement of plant resources, 18–20th December, Annamalai University, Annamalainagar, Tamil Nadu, India, p 108

Alam B, Das G, Raj S, Roy S, Pal TK, Dey SK (2003) Studies on yield and biochemical subcomponents of latex of rubber trees (*Hevea brasiliensis*) with special reference to the impact of low temperature in a non-optimal environment. J Rubb Res 6(4):241–257

Alam B, Nair DB, Jacob J (2005) Low temperature stress modifies the photochemical efficiency of a tropical tree species *Hevea brasiliensis*: Effect of varying concentrations of CO_2 and photon flux density. Photosynthetica 43(2):247–252

Alenius H, Kalkkinen N, Lukka M, Reunala T, Turjanmaa K et al (1995) Prohevein from the rubber tree (*Hevea brasiliensis*) is a major latex allergen. Clin Exp Aller 25(7):659–65

Alper H, Stephanopoulos G (2007) Global transcription machinery engineering: a new approach for improving cellular phenotype. Metab Eng 9(3):258–267

Ambily KK, Joseph M (2018) Effect of soil pH and base status on the growth of youngnatural rubber plants. Rubb Sci 31(3):232–242

Ambily KK, Mercy MA, Ravichandran S, Jessy MD (2020) Leaf potassium content as an index of adaptation to drought tolerance in natural rubber. Rubb Sci 33(2):210–220

An F, Li CZ, Zhang TT, Wang LF, Wang JK, Xie GS (2018) Effects of aluminum toxicity on physiological and leaf chlorophyll fluorescent characteristics of rubber tree seedlings. Ying Yong Sheng Tai Xue Bao 12:4191–4198

An Z, Zhao Y, Zhang X, Huang X, Hu Y, Cheng H, Li X, Huang H (2019) A high density genetic map and QTL mapping on growth and latex yield-related traits in *Hevea brasiliensis* Mull.Arg. Ind Crops Prod 132:440–448. https://doi.org/10.1016/j.indcrop.2019.03.002

Annamalainathan K, James J, Ravichandran S, Kumar S (2020) Water use by mature rubber trees in three different agro-climatic regions of India. Rubb Sci 33(2):140–151

Annamalainathan K, Joseph J Alam B, Satheesh PR, James J (2013) Seasonal changes in xylem sap flow rate in mature rubber plants. J Plantat Crops 41(3):343–349

Annamma Y, Marattukalam JG, Premakumari D,Saraswathy Amma CK, Licy J, Panikkar AO(1990). Promising rubber planting materials with special reference to Indian clones. In: Proceedings of planters conference, Kottayam, India, pp 62–70

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141(2):391–396

Asawatreratanakul K, Zhang YW, Wititsuwannakul D, Wititsuwannakul R, Takahashi S et al (2003) Molecular cloning, expression and characterization of cDNA encoding cis-prenyltransferases from *Hevea brasiliensis*: A key factor participating in natural rubber biosynthesis. Eur J Biochem 70(23):4671–4680

Baldwin JRJT (1947) *Hevea:* A First Interpretation: a cytogenetic survey of a controversial genus, with a discussion of its implications to taxonomy and to rubber production. J Hered 38(2):54–64

Bailey-Serres J, Parker JE, Ainsworth EA, Oldroyd GE, Schroeder JI (2019) Genetic strategies for improving crop yields. Nature 575(7781):109–118

Bennett MD, Leitch IJ (1997) Nuclear DNA amounts in angiosperms-583 new estimates. Ann Bot 80:169–196

Benson D, Lipman DJ, Ostell J (1993) GenBank. Nucleic Acids Res 21(13):2963–2965

Besse P, Seguin M, Lebrun P, Chevallier MH, Nicolas D, Lanaud C (1994) Genetic diversity among wild and cultivated populations of *Hevea brasiliensis* assessed by nuclear RFLP analysis. Theor Appl Genet 88(2):199–207

Bhaskar CVS, MohankrishnaT, Chandrashekar TR, Rao PS, Vijayakumar KR, Sethuraj MR (1991) Effect of irrigation on growth and other physiological parameters of *Hevea brasiliensis* in the Konkan region of India. Physiology and exploitation of *Hevea brasiliensis*.In: Proceedings of IRRDB symposium, Kunming,China,pp130–135

Blaukwill WJ(1989) The history of natural rubber production. In: Webster CC, Baulkwill WJ (eds) Rubber. Longman Scientific and Technical, Essex, UK, pp 1–56

Boghigian BA, Salas D, Ajikumar PK, Stephanopoulos G, Pfeifer BA (2012) Analysis of heterologous taxadiene production in K-and B-derived *Escherichia coli*. Appl Microbial Biotechnol 93(4):1651–1661

Brown D, Feeney M, Ahmadi M, Lonoce C, Sajari R, Di Cola A, Frigerio L (2017) Subcellular localization and interactions among rubber particle proteins from *Hevea brasiliensis*. J Exp Bot 68(18):5045–5055

Bushman BS, Scholte AA, Cornish K,Scott DJ, Brichta JL et al (2006) Identification and comparison of natural rubber from two *Lactuca* species. Phytochemistry 67(23):2590–2596

Buttery BR, Boatman SG (1976) Deficit and flow of latex. In: Kozlowski TT (ed) Water deficit and plant growth. Academic Press, New York, pp 233–289

Cao Y, Xiang X, Geng M, You Q, Huang X (2017) Effect of *HbDHN1* and *HbDHN2* genes on abiotic stress responses in arabidopsis. Front Plant Sci 8:470

Chandrasekhar TR, Jana MK, Thomas J, Vijayakumar KR, Sethuraj MR (1990) Seasonal changes in physiological characteristics and yield in newly opened trees of *Hevea brasiliensis* in North Konkan. Ind J Nat Rubb Res 3:88–97

Chanroj V, Rattanawong R, Phumichai T, Tangphatsornruang S, Ukoskit K (2017) Genome-wide association mapping of latex yield and girth in Amazonian accessions of *Hevea brasiliensis* grown in a suboptimal climate zone. Genomics 109(5–6):475–484

Chantuma AC, Kunarasiri A, Chantuma P (2012) Rubber new planting in Thailand: towards the world affected on climate change. Rubb Thai J1:40–47

Chao J, Chen Y, Wu S, Tian WM (2015) Comparative transcriptome analysis of latex from rubber tree clone CATAS8-79 and PR107. Genomics Data 5:120–121. http://doi.org/10.1016/j.gdata.2015.05.034

Chatzivasileiou A, Warda V, Edgara S, Stephanopoulos G (2018) Two-step pathway for isoprenoid synthesis. Proc Nat Acad Sci USA 116(2):506–511

Cheng H, Cai H, Fu H, An Z, Fang J et al (2015) Functional characterization of *Hevea brasiliensis* CRT/DRE binding factor 1 gene revealed regulation potential in the CBF pathway of tropical perennial tree. PLoS One 10:e0137634

Cheng H, Xia Y, Zhou J, Tang C, Huang H (2019) *Hevea* DB: a genetic resource database for rubber tree genomic study. bioRxivp.708305

Cheng H, Chen X, Huang H (2016a) The identification and expression analysis of cold responsive HbCOR47 gene from *Hevea brasiliensis*. Chin J Trop Crops 37:1924–1930

Cheng H, Chen X, Zhu J, Huang H (2016b) Overexpression of a *Hevea brasiliensis* ErbB-3 Binding protein 1 gene increases drought tolerance and organ size in Arabidopsis. Front Plant Sci 7:1703

Cheng T, Liu H, Zou H, Chen N, Shi M et al (2017) Enzymatic process optimization for the in vitro production of isoprene from mevalonate. Micro Cell Fact 16:8

Chiang CK, Xie W, McMahan C, Puskas JE (2011) Unraveling the mystery of natural rubber biosynthesis. Part I: investigation of the composition and growth of in vitro natural rubber using high resolution size exclusion chromatography. Rubb Chem Tech 84(2):166–177

Chinnusamy V, Zhu J, Zhu JK (2007) Cold stress regulation of gene expression in plants. Trends Plant Sci 12(10):444–451

Chow KS, Mat-Isa MN, Bahari A, Ghazali AK et al (2012) Metabolic routes affecting rubber biosynthesis in *Hevea brasiliensis* latex. J Exp Bot 63(5):1863–1871

Chow KS, Wan KL, Isa MNM, Bahari A, Tan SH et al (2007) Insights into rubber biosynthesis from transcriptome analysis of *Hevea brasiliensis* latex. J Exp Bot 58(10):2429–2440

Chrestin H, Gidrol X, Kush A (1997) Towards a latex molecular diagnostic of yield potential and the genetic engineering of the rubber tree. Euphytica 96(1):77–82

Clement-Demange A, Legnate H, Seguin M (2000) Rubber tree. Collection reperes. In: Charrier A, Hamon M, Hamon S, Nicolas D (eds) Tropical plant breeding. CIRAD-ORSTOM, Montpellier, France Tropical Plant Breeding. Sci Publ Inc. CIRAD-ORSTOM, Montpellier, France, pp 455–480

Clément-Demange A, Legnaté H, Seguin M,Carron M, Le Guen et al (2001) Rubber tree. In: Charrier A, Hamon M, Hamon S, Nicolas D (eds) Tropical plant breeding. CIRAD-ORSTOM, Montpellier, France Tropical Plant Breeding. Sci Publ Inc 3, pp 455–80

Clément-Demange A, Prapan K, Rattanawong R, Teerawatanasuk K (2006) Molecular genetic markers and rubber breeding in Thailand: 2—field study of the family RRIM600 x PB217 for QTL identification. In: Franco–Thai project 2005–2008: towards the improvement of rubber tree productivity, 1–2nd June, Bangkok, Thailand, p 21

Clément-Demange A, Rattanawong R, Prapan K, Lekawipat N, Teerawatanasuk K et al (2008) Identification de 2 QTLs explicatifs de la croissance et de la production de latex chez *Hevea brasiliensis*. In: Biologie Moléculaire des Ligneux, BML10, Nancy, pp 5–7

Conson AR, Taniguti CH, Amadeu RR,Andreotti IAA, de Souza LM et al (2018) High-resolution genetic map and QTL analysis of growth-related traits of *Hevea brasiliensis* cultivated under suboptimal temperature and humidity conditions. Front Plant Sci 9:1255

Cornish K (2001a) Biochemistry of natural rubber, a vital raw material, emphasizing biosynthetic rate, molecular weight and compartmentalization, in evolutionarily divergent plant species. Nat Prod Rep 18(2):182–189

Cornish K (2001b) Similarities and differences in rubber biochemistry among plant species. Phytochemistry 57(7):1123–1134

Cornish K, Blakeslee J (2011) Rubber biosynthesis in plants. The AOCS Lipid Library. http://lipidlibrary.aocs.org/plantbio/plantlip.html

Cornish K, Castillón J, Chapman MH (1998) Membrane-bound cis-prenyl transferase activity: regulation and substrate specificity. Biochemical principles and mechanisms of biosynthesis and biodegradation of polymers. Wiley-VCH-Verlag, pp 316–23

Cornish K, Castillón J, Scott DJ (2000) Rubber molecular weight regulation, *in vitro*, in plant species that produce high and low molecular weights *in vivo*. Biomacromol 1(4):632–641

Cornish K, Siler DJ (1995) Effect of different allylic diphosphates on the initiation of new rubber molecules and on cis-1, 4-polyisoprene biosynthesis in guayule (*Parthenium argentatum* Gray). J Plant Physiol 147(3–4):301–305

Dai L, Kang G, Li Y, Nie Z, Duan C, Zeng R (2013) In-depth proteome analysis of the rubber particle of *Hevea brasiliensis* (para rubber tree). Plant Mole Boil 82(1–2):155–68

Dai L, Kang G, Nie Z, Li Y, Zeng R (2016) Comparative proteomic analysis of latex from *Hevea brasiliensis* treated with Ethrel and methyl jasmonate using iTRAQ-coupled two-dimensional LC–MS/MS. J Proteom 132:167–175

Dai L, Nie Z, Kang G, Li Y, Zeng R (2017) Identification and subcellular localization analysis of two rubber elongation factor isoforms on *Hevea brasiliensis* rubber particles. Plant Physiol Biochem 111:97–106

d'Auzac J, Jacob L (1989) The composition of latex from *Hevea brasiliensis* as a laticiferous cytoplasm. In: d'Auzac J, Jacob JL, Chrestin H (eds) Physiology of the rubber tree latex, CRC Press, Boca Raton, Florida, pp 59–96

Das A, Basu D, Heinrich G (2014) Rubber nanocomposites. In: Encyclopedia of polymeric nanometerials. Springer-Verlqag, Berlin, Heidelberg

Das G, Raj S, Pothen J, Sethuraj MR, Sinha TP, Sen-Mandi S (1998) Status of free radical and its scavenging system with stimulation in *Hevea brasiliensis*. Plant Physiol Biochem 25:47–50

Dean W (1987) Brazil and the struggle for rubber. Cambridge University Press, Cambridge, UK

de Goncalves PS, Paiva JR, Souza RA (1983) Retrospectiva e Atualidade do Melhoramento Genetico da Seringueira (*Hevea* spp.) no Brasil e em paises Asiatics. Manaus: EMBRAPA-CNPSD 2, p 10

DeJong JM, Liu Y, Bollon AP, Long RM, Jennewein S, Williams D et al (2006) Genetic engineering of taxol biosynthetic genes in *Saccharomyces cerevisiae*. Biotechnol Bioeng 93(2):212–224

Dennis MS, Light DR (1989) Rubber elongation factor from *Hevea brasiliensis*: identification, characterization, and role in rubber biosynthesis. J Biol Chem 264(31):18608–18617

Deng XM, Wu SH, Dai XM, Tian WM (2016) Expression analysis of MVA and MEP metabolic pathways genes in latex and suspension cells of *Hevea brasiliensis*. Guihaia 36:449–455

Deng Z, Chen J, Wei Y, Liu H, Yang H, Dai L, Li D (2018) Two translationally controlled tumour protein (TCTP) genes from Hevea brasiliensis play overlapping and different roles in development and stress response. Ind Crops Prod 114:137–145

Devakumar AS, Prakash PG, Sathik MB, Jacob J (1999) Drought alters the canopy architecture and micro-climate of *Hevea brasiliensis* trees. Trees 13(3):161–167

Dey SK, Vijayakumar KR, Nair DB, Subramanian P (1999) Effect of temperature and vapor pressure on major yield components of rubber in humid and dry sub-humid climatic regions. Indian J Nat Rubb Res 11(1&2):69–76

Dijkman MJ (1951) *Hevea*: thirty years of research in the far east.329.coral Gables, FL, University of Miami Press

Doudna JA, Charpentier E (2014)The new frontier of genome engineering with CRISPR-Cas9. Science 28:346(6213)

Duan C, Argout X, Gebelin V, Summo M, Dufayard JF, Leclercq J, Hadi K, Piyatrakul P, Pirrello J, Rio M, Champion A, Montoro P (2013) Identification of the *Hevea brasiliensis* AP2/ERF superfamily by RNA sequencing. BMC genomics 14:30

El-Kassaby YA, Lstibůrek M (2009) Breeding without breeding. Genet Res (Camb)91(2):111–120. https://doi.org/10.1017/S0016672309000070X. PMID: 19393127

Fan Y, Xin S, Dai X, Yang X, Huang H, Hua Y (2020) Efficient genome editing of rubber tree (*Hevea brasiliensis*) protoplasts using CRISPR/Cas9 ribonucleoproteins. Ind Crops Prod 146:112146. https://doi.org/10.1016/j.indcrop.2020.112146

Faridah Y (1996) Changes in some physiological latex parameters in relation to overexploitation and the onset of induced tapping dryness. J Nat Rubb Res 10:182–198

FaridahYS AMA, Ghandimath H (1996) Changes in some physiological latex parameters in relation to over exploitation and onset of induced tapping panel dryness. J Nat Rubb Res 10:182–186

Farmer WR, Liao JC (2000) Improving lycopene production in *Escherichia coli* by engineering metabolic control. Nat Biotechnol 18:533–537

Feng SP, Li WG, Yu F, Wang JY, Wu YT (2010) Construction of genetic linkage map for rubber tree (*Hevea brasiliensis*) based on SSR markers. Hereditas 32(8):857–863

Finnegan EJ, Genger RK, Peacock WJ, Dennis ES (1998) DNA methylation in plants. Annu Rev Plant Physiol Plant Mol Biol 49:223–247 Gébelin V, Leclercq J, Kuswanhadi, Argout X, ChaidamsariT et al (2013a)The small RNA profile in latex from *Heveabrasiliensis* trees is affected by tapping panel dryness. Tree Physiol33(10):1084-98

Gébelin V, Argout X, Engchuan W, Pitollat B, Duan C et al (2012) Identification of novel microRNAs in *Hevea brasiliensis* and computationalprediction of their targets. BMC Plant Biol 12:18

Gébelin V, Leclercq J, Kuswanhadi, Argout X, Chaidamsari T et al (2013a)The small RNA profile in latex from *Heveabrasiliensis* trees is affected by tapping panel dryness. Tree Physiol 33(10):1084–1098

Gébelin V, Leclercq J, Hu S, Tang C, Montoro P et al (2013b) Regulation of MIR genes in response to abiotic stress in *Hevea brasiliensis*. Intl J Mol Sci 14(10):19587–19604

Gidrol X, Chrestin H, Tan HL, Kush A (1994) Hevein, a lectin-like protein from *Hevea brasiliensis* (rubber tree) is involved in the coagulation of latex. J Biol Chem 269(12):9278–9283

Goncalves PD, de Pavia JR (1983) Retrospectiva e atualidade do melhoramento genético da seringueira (*Hevea* spp.) no Brasil e em países asiáticos. Embrapa Amazônia Ocidental-Documentos (INFOTECA-E)

Gonçalves PD, Cardoso M, Ortolani AA (1990) Origin, variability and domestication of *Hevea*-a review. Pesquisa Agropecuaria Brasileira 25(2):135–156

Gong XX, Yan BY, Hu J, Yang CP, Li YJ et al (2018) Transcriptome profiling of rubber tree (*Hevea brasiliensis*) discovers candidate regulators of the cold stress response. Genes Genomi 40(11):1181–1197

Gonzalez AI, Saiz A, Acedo A, Ruiz ML et al (2013) Analysis of genomic DNA methylation patterns in regenerated and control plants of rye (*Secale cereale* L.). Plant Growth Regul 70:227–236

Greek BF (1991) Rubber demand is expected to grow after 1991. C & E News 69:37–54

Habib MA, Yuen GC, Othman F, Zainudin NN, Latiff AA, Ismail MN (2016) Proteomics analysis of latex from *Hevea brasiliensis* (clone RRIM 600). Biochem Cell Biol 95(2):232–242

Han KH, Shin DH, Yang J, Kim IJ, Oh SK, Chow KS (2000) Genes expressed in the latex of *Hevea brasiliensis*. Tree Physiol 20(8):503–510

Ho CY, Khoo SK, Meignanaratnam K, Yoon PK (1983) Potential new clones from mother tree selection. In: Proceedings of the rubber research institute of malaysia planters, conference 17–19th October, Kuala Lumpur, pp 201–218

Huand ZD, Pan YQ (1992) Rubber cultivation under climatic stress in China. In: Sethuraj MR, Mathew NM (eds) Natural Rubber: biology, cultivation and technology. Elsevier, Amsterdam, pp 220–238

Immethun CM, Hoynes O, Connor AG, Balassy A, Moon TS (2013) Microbial production of isoprenoids enabled by synthetic biology. Front Microbiol 4(75):1–8

Isarangkool Na Ayutthaya S, Do FC, Pannengpetch K, Junjittakarn J, Maeght JL et al (2009) Transient thermal dissipation method of xylem sap flow measurement: multi-species calibration and field evaluation. Tree Physiol 30(1):139–148

Jacob J, Annamalainathan K, Alam B, Sathik M, Thapliyal A, Devakumar A (1999a) Physiological constraints for cultivation of *Hevea brasiliensis* in certain unfavourable agroclimatic regions of India. Indian J Nat Rubb Res 12:1–16

Jacob J, Annamalainathan K, Alam B, Sathik MB, Tapliyal AP, Devakumar AS et al (1999b) Physiological constrains for cultivation of *Heveabrasiliensis* in certain unfavorable agroclimatic regions of India. Indian J Nat Rubb Res 12:1–16

Jacob J, Othman R, Mydin KK, Lam VL, Tu LM et al (2013) International clone exchange and genetic enhancement research in *Hevea brasiliensis*. Rubb Sci 26(1):1–2

Jacob J, Reju MJ, Meenakumari T, Gireesh T (2021) Law of diminishing marginal returns and yield ceiling in natural rubber. Rubb Sci 34(1):1–15

Jayashree R, Rekha K, Venkatachalam P, Uratsu SL, Dandekar AM et al (2003) Genetic transformation and regeneration of rubber tree (*Hevea brasiliensis* Muell. Arg) transgenic plants with a constitutive version of an anti-oxidative stress superoxide dismutase gene. Plant Cell Rep 22(3):201–209

Jayashree R, Nazeem PA, Venkatachalam P, Rekha K, Sreelatha S et al (2015) Integration and expression of *hmgr1* gene in the transgenic plants of *Hevea brasiliensis* (clone RRII 105). In: International Symposium on Plantation Crops, Kozhikode, Kerala, India

Jessy MD, Krishnakumar R, Annamalainathan K, Jacob J (2011) Climate uncertainties and early establishment of young rubber plants in traditional rubber growing regions of India. Nat Rubb Res 24(1):145–147

Jessy MD, Prasannakumari P, Jacob J (2014) Agromanagement techniques to mitigate drought in young rubber plantations. Rubb Sci 27(1):54–60

Jiang A (1998) Climate and natural production of rubber (*Hevea brasiliensis*) in Xishuangbanna, southern part of Yunnan province China. Intl J Biometeorol 32(4):280–282

Jinagool W, Rattanawong R, Sangsing K, Barigah ST, Gay F et al (2015) Clonal variability for vulnerability to cavitation and other drought-related traits in *Hevea brasiliensis* Müll. Arg. J Plant Hydraul 2:e001

Jones KL, Kim SW, Keasling JD (2000) Low-copy plasmids can perform as well as or better than high copy plasmids for metabolic engineering of bacteria. Metabolic Eng 2(4):328–338

Joseph (2011) Exchange properties of soils in the traditional rubber growing tract in South India. Nat Rub Res 24(1):97–105

Joseph (2016) Rubber growing soils of India: an overview. Rubber Sci 29(2):119–139

Kala RG, Kumari Jayasree P, Sobha S, Sushama Kumara S, Jayashree R et al (2003) Introduction of the gene coding for isopentenyl transferase into *Hevae brasiliensis*: effect on plant regeneration. In: Proceedings of the 10th congress of FAOBMB, December, pp 7–11

Kala RG, Jayasree R, Uratsu P, Dandekar AM, Thulaseedharan A (2019) Isopentenyl transferase gene incorporated GM rubber: effect on plant regeneration. In: Natural rubber industry: way forward for the competitiveness and sustainability: international rubber conference, Naypyi Taw, Myanmar, 30 Sep–1 Oct 2019

Karthikakuttyamma M, Nair ANS, Krishnakumar AK, Potty SN, Mathew M (1991) Importance of inorganic phosphorous fraction in rubber growing soils. Indian J Nat Rubb Res 4(1):72–76

Karthikakuttyamma M, Joseph M, Nair ANS (2000) Soil and nutrition. In: George PJ, Jacob CK (eds) Natural rubber. Agromanagement and crop processing rubber research institute of India, Kottayam, pp 170–198

Karyudi N (2004) Variation of osmoregulation in *Hevea* rubber clones in response to water stress. In: Proceedings of the IRRDB conference, Kunming, Yunnan, China, pp 317–323

Kharche VK, Sehgal JL, Challa O (1995) Evaluation of soil-site conditions for suitability of rubber. Agropedology 5:69–78

Khoo SK, Yoon PK, Meignanaratnam K(1982) Early results of mother-tree (ortet) selection [breeding, *Hevea* rubber, Malaysia]. Planters' Bulletin Rubber Research Institute of Malaysia (Malaysia), pp 33–49

Kim Y, Wyslouzil BE, Weathers PJ(2002) Secondary metabolism of hairy root cultures in bioreactors. In Vitro Cell Dev Biol –Plant 38(1):1–10

Kim IJ, Ryu SB, Kwak YS, Kang H (2004) A novel cDNA from *Parthenium argentatum* Gray enhances the rubber biosynthetic activity *in vitro*. J Exp Bot 55(396):377–385

Klein-Marcuschamer D, Ajikumar PK, Stephanopoulos G (2007) Engineering microbial cell factories for biosynthesis of isoprenoid molecules: beyond lycopene. Trends Biotechnol 25(9):417–424

Korman TP, Sahachartsiri B, Li D, Vinokur JM, Eisenberg D, Bowie JU (2014) A synthetic biochemistry system for the *in vitro* production of isoprene from glycolysis intermediates. Protein Sci 23(5):576–585

Kumar S, Hahn FM, Baidoo E, Kahlon TS, Wood DF et al (2012) Remodeling the isoprenoid pathway in tobacco by expressing the cytoplasmic mevalonate pathway in chloroplasts. Metab Eng 14(1):19–28

Kuruvilla L, Sathik MB, Thomas M, Luke LP, Sumesh KV, Annamalainathan K (2016) Expression of miRNAs of *Hevea brasiliensis* under drought stress is altered in clones with varying levels of drought tolerance. Indian J Biotechnol 15(2):153–160

Kuruvilla L, Sathik MM, Thomas M, Luke LP, Sumesh KV (2017) Identification and validation of cold responsive microRNAs of *Heveabrasiliensis* using high throughput sequencing. J Crop Sci Biotechnol 20(5):369–377

Labra M, Ghiani A, Citterio S, Sgorbati S, Sala F, Vannini C, Bracale M (2002) Analysis of cytosine methylation pattern in response to water deficit in pea root tips. Plant Biol 4(6):694–699

Laffaldano BJ, Zhang Y, Cornish K (2016) CRISPR/Cas9 genome editing of rubber producing dandelion Taraxacum kok-saghyz using *Agrobacterium rhizogenes* without selection. Ind Crops Prod 89:356–362

Lam LV, Thanh T, Trang LTT, Truong VV, Lam HB (2012) *Hevea* germplasm in Vietnam: conservation, characterization, evaluation and utilization. In: Caliskan M (ed) Genetic diversity in plants. InTech Publisherp, Rijeka, pp 433–456

Lau N, Makita Y, Kawashima M, Taylor T, Kondo S, Othman A et al (2016) The rubber tree genome shows expansion of gene family associated with rubber biosynthesis. Sci Rep 6(1):1–14 Article No 28594

Le Guen V, Garcia D, Doaré F, Mattose CRR, Condina V et al (2011a) A rubber tree's durable resistance to Microcyclus ulei is conferred by a qualitative gene and a major quantitative resistance factor. Tree Genet Genomes 7:877–889

Leclercq J, Martin F, Sanier C, Clément-Vidal A, Fabre D et al (2012) Over-expression of a cytosolic isoform of the HbCuZnSOD gene in *Hevea brasiliensis* changes its response to a water deficit. Plant Mol Biol 80(3):255–272

Leitch AR, Lim KY, Leitch IJ, O'Neill M, Chye M, Low F (1998) Molecular cytogenetic studies in rubber, *Hevea brasiliensis* Muell. Arg. (Euphorbiaceae). Genome 41:464–467

Lekawipat N, Teerawatanasuk K, Vanavichit A, Toojinda T, Tragoonrung S (2003) Evaluating the genetic relatedness of wild and cultivated *Heveabrasiliensis* accessions with SSCP Markers. SABRAO J Breed Genet 35:123–134

Lertpanyasampatha M, Gao L, Kongsawadworakul P, Viboonjun U, Chrestin et al (2012) Genome-wide analysis of microRNAs in rubber tree (*Hevea brasiliensis* L.) using high-throughput sequencing. Planta 236(2):437–445

Lespinasse D, Rodier-Goud M, Grivet L, Leconte A, Legnaté H, Seguin M (2000)A saturated genetic linkage map of rubber tree (*Hevea* spp.) based on RFLP, AFLP, microsatellite, and isozyme markers. Theor Appl Genet 100(1):127–138

Lestari R, Rio M, Martin F, Leclercq J, Leclercq J, Woraathasin, N et al (2018) Overexpression of *Hevea brasiliensis* ethylene response factor Hb ERF-IX c5enhances growth and tolerance to abiotic stress and affects laticifer differentiation. Plant Biotechnol J 6(1):322–336

Le Guen V, Garcia D, Doaré F, Mattos CRR, Condina V et al (2011b) A rubber tree's durable resistance to *Microcyclus ulei* is conferred by a qualitative gene and a major quantitative resistance factor. Tree Genet Genomes 7:877–889

Li D, Deng Z, Liu X, Qin B (2013) Molecular cloning, expression profiles and characterization of a novel translationally controlled tumor protein in rubber tree (*Hevea brasiliensis*). J Plant Physiol 170:497–504. https://doi.org/10.1016/j.jplph.2012.11.014

Li D, Hao L, Liu H, Zhao M, Deng Z et al (2015) Next-generation sequencing, assembly, and comparative analyses of the latex transcriptomes from two elite *Hevea brasiliensis* varieties. Tree Genet Genomes 11(5):1–9

Li D, Wang X, Deng Z, Liu H, Yang H, He G (2016a) Transcriptome analyses reveal molecular mechanism underlying tapping panel dryness of rubber tree (*Hevea brasiliensis*). Sci Rep 6:23540. https://doi.org/10.1038/srep23540

Li D, Zeng R, Li Y, Zhao M, Chao J, Li Y, Wang K, Zhu L, Tian WM, Liang C (2016b) Gene expression analysis and SNP/InDel discovery to investigate yield heterosis of two rubber tree F1 hybrids. Sci Rep 6(1):1–12. https://doi.org/10.1038/srep24984

Licy J, Saraswathyamma CK, Premakumari D, Meenakumari T, Meenatoor JR, Nazeer MA (2003) Genetic parameters and heterosis in rubber (*Hevea brasiliensis*) Muell Arg. V. Hybrid vigour for yield and yield components among the RRII 400 series clones in small scale cultivation. Indian J Nat Rubb Res 16(1&2):75–80

Lin Y, GraceJ ZW, Dong Y (2018) Water-use efficiency and its relationship with environmental and biological factors in a rubber plantation. J Hydrol 563:273–282

Liu W, Hu H, Ma Y, Li H (2006) Environmental and socioeconomic impacts of increasing rubber plantations in Menglun Township. Res Dev 26(3):245–253

Liu JP, Xia ZQ, Tian XY, Li YJ (2015) Transcriptome sequencing and analysis of rubber tree (*Hevea brasiliensis* Muell.) to discover putative genes associated with tapping panel dryness (TPD). BMC Genomics 16:398

Liu JP, Zhuang YF, Guo XL, Li YJ (2016) Molecular mechanism of ethylene stimulation of latex yield in rubber tree (*Hevea brasiliensis*) revealed by de novo sequencing and transcriptome analysis. BMC Genomics 17(1):1–13. https://doi.org/10.1186/s12864-016-2587

Liu J, Shi C, Shi CC, Li W, Zhang QJ et al (2020) The chromosome-based rubber tree genome provides new insights into spurge genome evolution and rubber biosynthesis. MolPlant 13(2):336–350

Long X, He B, Fang Y, Tang C (2016) Identification and characterization of the glucose-6-phosphate dehydrogenase gene family in the para rubber tree *Hevea Brasiliensis*. Front Plant Sci 25(7):215
Luke LP, Sathik MMB, Thomas M, Kuruvilla L, Sumesh KV, Annamalainathan K (2015) Quantitative expression analysis of drought responsive genes in clones of *Hevea* with varying levels of drought tolerance. Physiol Mol Biol Plants 21(2):179–86
Luo H, van Coppenolle B, Seguin M, Boutry M (1995) Mitochondrial DNA polymorphism and phylogenetic relationships in *Hevea brasiliensis*. Mol Breed 1:51–63
Ma X, An F, Wang L, Guo D, Xie G, Liu Z (2020) Genome-wide identification of aluminum-activated malate transporter (ALMT) gene family in rubber trees (*Hevea brasiliensis*) highlights their involvement in aluminum detoxification. Forests 11(2):142
Mai J, Herbette S, Vandame M, Cavaloc E, Julien JL et al (2010) Contrasting strategies to cope with chilling stress among clones of a tropical tree, *Hevea brasiliensis*. Tree physiol 30(11):1391–402
Majumder SK (1964) Chromosome studies in some species of *Hevea*. J Rubb Res Inst, Malaya 18:269
Manmuen S, Chantuma A, Teerawatanasuk K, Knowe S (1993) Growth characteristic of rubber in the drought. Para Rubber Bull Thailand 13:12–30
Mantello CC, Cardoso-Silva CB, da Silva CC, de Souza LM, Scaloppi Junior EJ et al (2014) De novo assembly and transcriptome analysis of the rubber tree (*Hevea brasiliensis*) and SNP markers development for rubber biosynthesis pathways. PLoS One 219(7):e102665
Martin VJ, Pitera DJ, Withers ST, Newman JD, Keasling JD (2003) Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. Nat Biotechnol 21(7):796–802
Mathew R, Roy B, Ravindran M, Nazeer M, Saha T (2005) Phylogenetic relationship of *Hevea* species as revealed through molecular markers. Natl Rubb Res 18:14–24
Meenattoor JR, Sasikumar B, Soman TA, Gupta CK, Meti S et al (2000) Genotype x environment interactions in *Hevea* in diverse agroclimatic conditions in India: Preliminary growthresults. In: Pushparajah E (ed) Plantation tree crops in the New Millennium: the way ahead. The Incorporated Society of Planters, Kuala Lumpur, Malaysia, p 183
Meenattoor JR, Vinod KK, Krishnakumar AK, Sethuraj MR, Potty SN, Sinha RR (1991) Clone x environment interaction during early growth phase of *Hevea brasiliensis*. I. Clonal stability on girth. Indian J Nat Rubb Res 4(1):51–4
Meti S, Meenattoor R, Mondal GC, Chaudhuri D (2003) Impact of cold weather condition on the growth of *Hevea brasiliensis* clones in North West Bengal. J Nat Rubb Res16(1&2):53–59
Merkulov S, van Assema F, Springer J, Fernandez Del Carmen A, Mooibroek H (2000) Cloning and characterization of the *Yarrowia lipolytica* squalene synthase (SQS1) gene and functional complementation of the *Saccharomyces cerevisiaeerg9* mutation. Yeast 16:197–206
Miao Z, Gaynor JJ (1993) Molecular cloning, characterization and expression of Mn-superoxide dismutase from the rubber tree (*Hevea brasiliensis*). Plant Mol Biol 23(2):267–277
Mondal GC, Das K, Singh RP, Mandal D, Gupta C et al (1999) Performance of *Hevea* clones in Assam. Indian J Nat Rubb Res 12(1&2):55–61
Montoro P, Wu S, Favreau B, Herlinawati E, Labrune C, Martin Magniette ML, Pointet S, Rio M, Leclercq J, Ismawanto S, Kuswanhadi (2018) Transcriptome analysis in *Hevea brasiliensis* latex revealed changes in hormone signalling pathways during ethephon stimulation and consequent tapping panel dryness. Sci Rep 8(1). https://doi.org/10.1038/s41598-018-26854-y
Mydin KK (2014) Genetic improvement of *Hevea brasiliensis*: sixty years of breeding efforts in India. Rubb Sci 27(2):153–181
Mydin KK (2011) Polycross breeding towards evolving genetically diverse *Hevea* clones for sustainability. In: IRRDB international rubber conference,15–16th December Chiang Mai,Thailand, pp 1–10
Mydin KK, John A, Meenakumari T (2016) Clones evolved by ortet selection from polycross populations of rubber in Central India. Rubb Sci 29(3):246–255
Oh SK, Kang H, Shin DH, Yang J, Chow KS et al (1990) Isolation, characterization, and functional analysis of a novel cDNA clone encoding a small rubber particle protein from *Hevea brasiliensis*. J Biol Chem 274(24):17132–17138

Ong SH (1975) Chromosome morphology at the pachytene stage in *Hevea brasiliensis*-a preliminary report. In:International rubber conference, 20th Oct, Kuala Lumpur (Malaysia), pp 3–12

Ouseph T (1987) Drought and the rubber planting. Rubber Board Bull 22(4):24–26

Paardekooper EC, Sookmark SO (1969) Diurnal variation in latex yield and dry rubber content and relation to saturation deficit of air. Rubb Res Inst Malaya J 21(3):341–347

Pakianathan SW, Haridas G, d' Auzac J (1989) Water relation and latex flow. In: d' Auzac J, Jacob JL, Chertin H (eds) Physiology of rubber tree latex. CRC Press, Boca Raton, pp 233–256

Pellicer J, Fay MF, Leitch IJ (2010) The largest eukaryotic genome of them all. Bot J Linn Soc 164(1):10–15

Pootakham W, Chanprasert J, Jomchai N, Sangsrakru D, Yoocha T et al (2011) Single nucleotide polymorphism marker development in the rubber tree, *Hevea brasiliensis* (Euphorbiaceae). Am J Bot 98:337–338

Pootakham W, Ruang-Areerate P, Jomchai N, Sonthirod C, Sangsrakru D, Yoocha T et al (2015) Construction of a high-density integrated genetic linkage map of rubber tree (*Hevea brasiliensis*) using genotyping-bysequencing (GBS). Front Plant Sci 6:367

Pootakham W, Sonthirod C, Naktang C, Ruang-Areerate P, Yoocha T et al. (2017) *De novo* hybrid assembly of the rubber tree genome reveals evidence of paleotetraploidy in *Hevea* species. Sci Rep 7:41457

Post J, van Deenen N, Fricke J, Kowalski N, Wurbs D et al (2012) Laticifer-specific *cis-prenyltransferase* silencing affects the rubber, triterpene, and inulin content of *Taraxacum brevicorniculatum*. Plant Physiol 58(3):1406–1417

Priyadarshan PM (2003) Breeding *Hevea brasiliensis* for environmental constraints. Adv Agron 79:352–400

Priyadarshan PM (2016) Genetic diversity and erosion in *Hevea* Rubber. In: Ahuja MR, Jain MS (eds) Genetic diversity and erosion in plants. Springer, pp 233–267

Priyadarshan PM (2017) Biology of *Hevea* Rubber. Springer International, Berlin, Germany, ISBN 9783319545066

Priyadarshan PM, de Gonçalves PS (2002) *Hevea* gene pool for breeding. Genet Resour Crop Evol (50):101–114

Priyadarshan PM, Clément-Demange A (2004) Breeding *Hevea* rubber: formal and molecular genetics. Adv Genet 52:51–116

Priyadarshan PM, Hoa TT, Huasun H, De Gonçalves P (2005) Yielding potential of rubber (*Hevea brasiliensis*) in sub-optimal environments. J Crop Improv 4(1–2):221–247

Qu Y, Chakrabarty R, Tran HT, Kwon EJG, Kwon M et al (2015) A lettuce (*Lactuca sativa*) homolog of human Nogo-B receptor interacts with cis-prenyltransferase and is necessary for natural rubber biosynthesis. J Biol Chem 290(4):1898–1914

Rahman AY, Usharraj AO, Misra BB, Thottathil GP, Jayasekaran K et al (2013) Draft genome sequence of the rubber tree *Hevea brasiliensis*. BMC Genomics 14(1):1–5

Ramaer H (1935) Cytology of *Hevea*. Genetics 17:193–194

Rao PS, Jayaratnam K, Sethuraj MR (1990) Water balance studies of the rubber growing regions of South India. J Appl Hydrol 3(2):23–30

Rao PS, Saraswathyamma CK, Sethuraj MR (1998a) Studies on the relationship between yield and meteorological parameters of para rubber tree (*Hevea brasiliensis*). Agric Meteorol 90(3):235–244

Rao G, Devakumar A, RajagopaL R, Annamma Y, Vijayakumar K, Sethuraj MR (1988b) Clonal variation in leaf epicuticular waxes and reflectance: possible role in drought tolerance in *Hevea*. Indian J Nat Rubb Res 1:84–87

Rattanawong R, Prapan K, Lekawipat N, Teerawatanasuk K, Kasemsap P, Seguin M, Clement Demange A (2008) QTLs detection for growth and initial latex production in rubber (*Hevea brasiliensis*). In: IRRDB, Kuala Lumpur, 13–14 October

Rekha K, Jayashree R, Sushamakumar S, Sobha S, Supriya R, Nazeem PA (2013) Integration and expression of osmotin gene in *Hevea brasiliensis via Agrobacterium* mediated transformation. J Plantation Crops 41:80–85

Rekha K, Nazeem PA, Venkatachalam P, Jayashree R, Sobha S, Kumari SS (2014) Development of osmotin transgenics in *Hevea brasiliensis* Muell. Arg. using explants of zygotic origin. J Trop Agri 52(1):7–20

Rekha K, Uthup TK, Sankaran S, Unnikrishnan D, Saha T, Nair SS (2015) Genetic and epigenetic uniformity of polyembryony derived multiple seedlings of *Hevea brasiliensis*. Protoplasma 252(3):783–796

Rosa L, Rulli MC, Davis KF, Chiarelli DD, Passera C, D'Odorico P (2018) Closing the yield gap while ensuring water sustainability. Environ Res Lett 13(10):104002

Roy CB, Nazeer MA, Saha T (2004) Identification of simple sequence repeats in rubber (*Heveabrasiliensis*). Curr Sci 25:807–811

Roy CB, Liu H, Rajamani A, Saha T (2019) Transcriptome profiling reveals genetic basis of disease resistance against *Corynespora cassiicola* in rubber tree (*Hevea brasiliensis*). Curr Plant Biol 17:2–16. https://doi.org/10.1016/j.cpb.2019.02.002

Saha T, Priyadarshan PM (2012) Genomics of *Hevea* rubber. In: Schnell RJ, Priyadarshan PM (eds) Genomics of tree crops. Springer, Berlin, pp 261–298

Saha T, Roy CB, Nazeer MA (2005) Microsatellite variability and its use in the characterization of cultivated clones of *Hevea brasiliensis.* Plant Breed 124:86–89

Saha T, Alam B, Ravindran M, Thomas KU (2010) Cold-induced gene expression profiling in rubber (*Hevea brasiliensis*). In: Proceedings of the international workshop on climate change and rubber cultivation. RRII, Kottayam, India

Salgado LR, Koop DM, Pinheiro DG, Ronan R, Le Vincent G et al (2014) De novo transcriptome analysis of *Hevea brasiliensis* tissues by RNA-seq and screening for molecular markers. BMC Genomics15(1):1–5

Sankariammal L, Mydin KK, Thomas V, Varghese YA (2011) Heterosis for yield and growth in Wickham x Amazonian hybrids of *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg. Natl Rubb Res 24(2):187–196

Samarappuli L, Yogaratnam N (1998) Tolerance of rubber plantations to drought and atmospheric warming: a review. J Rubb Res Inst, Sri Lanka 81:51–67

Samarappuli L, Yogaratnam N, Hettiarachchi R (1992) Performance of some RRIC 100 series clones under different soil moisture regimes. J Rubb Res Inst Sri Lanka 72:49–61

Samarappuli L, Yogaratnam N, Karunadasa P, Mitrasena U (1993) Role of potassium on growth and water relations of rubber plants. J Rubb Res Inst Sri Lanka 73:37–57

Sandeep K, Das G, Mydin K, Kumar S (2015) Performance of some *Hevea* clones under the cold prone climate of sub-himalayan West Bengal. Rubb Sci 28:247–254

Sando T, Takeno S, Watanabe N, Okumoto H, Kuzuyama T et al (2008) Cloning and characterization of the 2-C-methylD-erythritol 4-phosphate (MEP) pathway genes of a natural-rubberproducing plant *Hevea brasiliensis.* Biosci Biotechnol Biochem 72:2903–2917

Satheesh PR, Jacob J (2011) Impact of climate warming on natural rubber productivity in different agro-climatic regions of India. Natl Rubb Res 24(1):1–9

Sathik MBM, Kuruvilla L (2017) Developments in research on abiotic stress responsive microRNAs of *Hevea brasiliensis.* Indian J Plant Physiol 22(4):470–483

Sathik MBM, Kuruvilla L, Thomas M, Luke LP, Satheesh PR et al (2012) Quantitative expression analysis of stress responsive genes under cold stress in *Hevea brasiliensis.* Rubb Sci 25(2):199–213

Sathik MBM, Luke LP, Rajamani A, Kuruvilla L, Sumesh KV, Thomas M (2018) De novo transcriptome analysis of abiotic stress-responsive transcripts of *Hevea brasiliensis.* Mol Breed 38(3):1–7

Schultes RE (1990)A brief taxonomic view of the genus *Hevea.* In: International rubber research and development board, monograph, no. 14, Kuala Lumpur, Malaysia, p 57

Scott DJ, da Costa BM, Espy SC, Keasling JD, Cornish K (2003) Activation and inhibition of rubber transferases by metal cofactors and pyrophosphate substrates. Phytochemistry 64(1):123–134

Schuster SC (2008) Next-generation sequencing transforms today's biology. Nat Methods 5:16–18

Seguin M, Lespinasse D, Rodier-Goud M, Legnate H, Troispoux V, Pinard F, Clement-Demange A (1996) Genome mapping in connection with resistance to the South American Leaf Blight in rubber tree (*Hevea brasiliensis*). In: Third ASAP conference on agricultural biotechnology, Hua-Hin, Thailand, 10–15 Nov 1996, vol 1, pp 1–8

Sena Gomes AR, Kozlowski TT (1988) Physiological and growth responses to flooding of seedlings of *Hevea brasiliensis*. Biotropica 20:286–293

Sethuraj MR (1986) Physiology of growth and yield in *Hevea brasiliensis*. In: Proceedings of international rubber conference. Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia

Sethuraj MR, Rao CG, Raghavendra AS (1984) The pattern of latex flow from rubber tree (*Hevea brasiliensis*) in relation to water stress. J Cell Biochem (suppl) 8B:236

Sethuraj MR, Potty SN, Vijayakumar KR, Krishnakumar AK, Rao PS et al (1989) Growth performance of *Hevea* in the non-traditional regions of India. In: Proceedings of rubber growers conference. Rubber Research Institute of Malaysia, Malacca, pp 212–227

Shafar JM, Noordin WD, Zulkefly S, Shamshudin J, Hanafi MM (2017) Improving soil chemical properties and growth performance of *Hevea brasiliensis* through basalt application. In: Proceedings of international rubber conference, pp 308–320. ejournal.puslitkaret.co.id

Shao Z, Luo Y, Zhao H (2012) DNA assembler method for construction of zeaxanthin-producing strains of Saccharomyces cerevisiae. Methods Mol Biol 898:251–262

Shearman JR, Sangsrakru D, Ruang-Areerate P, Sonthirod C, Uthaipaisanwong P et al (2015) Assembly and analysis of a male sterile rubber tree mitochondrial genome reveals DNA rearrangementevents and a novel transcript. BMC Plant Biol 14:45. https://doi.org/10.1186/1471-2229-14-45PM

Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotechnol 26(10):1135–1145. https://doi.org/10.1038/nbt1486 PMID: 18846087

Shi MJ, Hao BZ, Wu JL, Tian WM (2010) Progress in mechanism for the regulation of the laticifer wound plug formation in rubber tree (*Hevea brasiliensis* Muell. Ar**g.**). Chin J Trop Crops 11:2043–2050

Shiba Y, Paradise EM, Kirby J, Ro DK, Keasling JD (2007) Engineering of the pyruvate dehydrogenase bypass in Saccharomyces cerevisiae for high-level production of isoprenoids. Metab Eng 9(2):160–168

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6:410–417

Siler DJ, Goodrich-Tanrikulu M, Cornish K, Stafford AE, McKeon TA (1997) Composition of rubber particles of *Hevea brasiliensis*, *Parthenium argentatum*, *Ficus elastica*, and *Euphorbia lactiflua* indicates unconventional surface structure. Plant Physiol Biochem 36:881–890

Simmonds NW (1989) Rubber breeding. Rubber. Longman, London, pp 85–124

Smolke CD, Martin VJ, Keasling JD (2001) Controlling the metabolic flux through the carotenoid pathway using directed mRNA processing and stabilization. Metab Eng 3(4):313–321

Sobha S, Sushamakumari S, Thanseem I, Jayasree PK, Rekha K et al (2003) Genetic transformation of *Hevea brasiliensis* with the gene coding for superoxide dismutase with FMV 34S promoter. Curr Sci 25:1767–1773

Souza LM, Mantello CC, Suzuki F et al (2011) Development of a genetic linkage map of rubber tree (*Hevea brasiliensis*) based on microsatellite markers. BMC Proc 5:39. https://doi.org/10.1186/1753-6561-5-S7-P39

Souza LM, Gazaffi R, Mantello CC, Silva CC, Garcia D et al (2013) QTL mapping of growth-related traits in a full-sib family of rubber tree (*Hevea brasiliensis*) evaluatedin a sub-tropical climate. PLoS ONE 8(4):e61238

Sreelatha S, Simon SP, Kurup GM, Vijayakumar KR (2007) Biochemical mechanisms associated with low yield during stress in *Hevea* clone RRII 105. J Rubb Res 10:107–115

Sreelatha S, Mydin KK, Simon SP, Krishnakumar R, Jacob J, Annamalainathan K (2011) Seasonal variations in yield and associated biochemical changes in RRII 400 series clones of *Hevea brasiliensis*. Nat Rubb Res 24:117–123

Stephanopoulos G, Sinskey AJ (1993) Metabolic engineering-methodologies and future prospects. Trends Biotechnol 11(9):392–396

Storz G (2002)An expanding universe of noncoding RNAs. Science 296:1260–1263

Sumesh KV, Satheesh PR, Sreelatha S, Ravichandran S, Thulaseedharan A et al (2014) Drought tolerance in MnSOD transgenic *Hevea brasiliensis* in a dry sub-humid environment. J Plant Crops 42(1):70–77

Syamala VK, Mercykutty Joseph, Punnoose KI (2003) Effect of liming on soil properties and growth of rubber seedlings. Indian J of Nat Rub Res 16(1&2):66–74

Tan H (1987) Strategies in rubber tree breeding. In: Abbott AJ, Atkin RK (eds) Improving vegetatively propagated crops. Academic Press, London, pp 27–62

Tang C, Yang M, Fang Y, Luo Y, Gao S et al (2016) The rubber tree genome reveals new insights into rubber production and species adaptation. Nat Plants 2(6):1–10

Tangpakdee J, Tanaka Y, Ogura K, Koyama T, Wititsuwannakul R et al (1997) Rubber formation by fresh bottom fraction of *Hevea* latex. Phytochemistry 45(2):269–274

Triwitayakorn K, Chatkulkawin P, Kanjanawattanawong S, Sraphet S, Yoocha T, Sangsrakru D et al (2011) Transcriptome sequencing of *Hevea brasiliensis* for development of microsatellite markers and construction of a genetic linkage map. DNA Res 18:471–482

Ulaganathan A, Satisha GC, Karthikakuttyamma M, Ambily KK, Nair ANS (2005) Phosphorous fraction and fixation of added phosphorous in rubber growing soils of Kerala. Rubb Sci 18(1):31–37

Ule EH (1905) Die Kautschukpflanzen der Amazonas-Expedition und ihre Bedeutung fudie Pflanzengeographie (Therubber plants of the Amazonas Expedition and their importance for geography). Botanische Jahrbiicher fur systematic pflanzengesehichte und Pflanzengeographie 35:663–678

Uthup TK, Karumamkandathil R, Ravindran M, Saha T (2018) Heterografting induced DNA methylation polymorphisms in *Hevea brasiliensis.* Planta 248(3):579–589

Uthup TK, Ravindran M, Bini K, Thakurdas S (2011) Divergent DNA methylation patterns associated with abiotic stress in *Hevea brasiliensis.* Mol Plant 4(6):996–1013

Vadali RV, Fu Y, Bennett GN, San KY (2005) Enhanced lycopene productivity by manipulation of carbon flow to isopentenyl diphosphate in *Escherichia coli.* Biotechnol Prog 21(5):1558–61

Varghese YA, Knaak C, Sethuraj MR, Ecke W (1997) Evaluation of random amplified polymorphic DNA (RAPD) markers in *Hevea brasiliensis.* Plant Breed 116(1):47–52

Varghese YA, Mydin KK (2000) Genetic improvement. In: George PJ, Jacob CK (eds) Natural rubber: agromanagement and crop processing. Rubber Research Institute of India, pp 297–308

Venkatachalam P, Thomas S, Priya P Thanseem I, Gireesh T, Saraswathyamma CK,et al (2002) Identification of DNA polymorphism with the cultivated clones of rubber tree (*Hevea brasiliensis* Muell. Arg.). Indian J Natl Rubb Res 15:172–181

Venkatachalam P, Thulaseedharan, A, Raghothama K (2007) Identification of expression profiles of tapping panel dryness (TPD) associated genes from the latex of rubber tree (*Heveabrasiliensis* Muell. Arg.). Planta 226(2):499–515

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ et al (2001) The sequence of the human genome. Science 291(5507):1304–1351

Verheye W (2010) Growth and production of rubber. In: Verheye W (ed) Land use, land cover and soil science. UNESCO, EOLSS Publishers

Vijayakumar KR, Dey SK, Chandrasekhar TR, Devakumar A, Mohanakrishna T et al (1998) Irrigation requirement of rubber trees (*Hevea brasiliensis)* in the subhumid tropics. Agri Water Manag 35(3):245–259

Vinod KK, Meenattoor JR, Priyadarshan PM, Pothen J, Chaudhuri D et al (1996) Early performance of some clones of *Hevea brasiliensis* Muell. Arg. in Tripura. Indian J Natl Rubb Res 9(2):123–129

Wang C, Deng P, Chen L, Wang X, Ma H et al (2013) Wheat WRKY transcription factor TaWRKY10 confers tolerance to multiple abiotic stresses in transgenic tobacco. PLoS ONE 8(6):e65120

Wang J, Yang Y, Liu X, Huang J, Wang Q, Gu J et al (2014) Transcriptome profiling of the cold response and signaling pathways in *Lilium lancifolium.* BMC Genomics 15(1):1–20

Wang LF (2014) Physiological and molecular responses to drought stress in rubber tree (*Hevea brasiliensis* Muell. Arg.). Plant Physiol Biochem 83:243–249
Wei Y, Lee JM, Richmond C, Blattner FR, Rafalski JA, Larossa RA (2001) High-density microarray mediated gene expression profiling of *Escherichia coli*. J Bacteriol 183:545–556
Wichitchonchai N, Manmuen S (1992) Yield of rubber in the North Eastern provinces. Para Rubber Bull Thailand 12:81–101
Wycherley PR (1968) Introduction of *Hevea* to the orient. Planter 4:1–11
Wycherley PR (1976) Rubber. In: Simmonds NW (ed) Evolution of crop plants. Longman, London, pp 77–80
Xia Z, Liu K, Zhang S, Yu W, Zou M et al (2018) An ultra-high density map allowed for mapping QTL and candidate genes controlling dry latex yield in rubber tree. Ind Crops Prod 120:351–356
Xia Z, Xu H, Zhai J, Li D, Luo H et al (2011) RNA-Seq analysis and de novo transcriptome assembly of *Hevea brasiliensis*. Plant Mol Biol 77(3):299–308
Xiang Q, Xia K, Dai L, Kang G, Li Y et al (2012) Proteome analysis of the large and the small rubber particles of *Hevea brasiliensis* using 2D-DIGE. Plant Physiol Biochem 60:207–213
Xie W, McMahan CM, Degraw AJ, Distefano MD, Cornish K et al (2008) Initiation of rubber biosynthesis: *In vitro* comparisons of benzophenone-modified diphosphate analogues in three rubber-producing species. Phytochemistry 69(14):2539–2545
Yamashita S, Yamaguchi H, Waki T, Yuichi A, Makie M et al (2016) Identification and reconstitution of the rubber biosynthetic machinery on rubber particles from *Hevea brasiliensis*. elife 5:e19022
Yan Y, Chemler J, Huang L, Martens S, Koffas MA (2005) Metabolic engineering of anthocyanin biosynthesis in *Escherichia coli*. Appl Environl Microbiol 71(7):3617–3623
Yang H, Dai L, Wei Y, Deng Z, Li D (2019) Melatonin enhances salt stress tolerance in rubber tree (*Hevea brasiliensis*) seedlings. Ind Crops Prod 145:111990
Yeang HY, Arif SA, Yusof F, Sunderasan E (2002) Allergenic proteins of natural rubber latex. Methods 27(1):32–45
Yuan LZ, Rouvière PE, La Rossa RA, Suh W (2006) Chromosomal promoter replacement of the isoprenoid pathway for enhancing carotenoid production in *E. coli*. Metab Eng 8(1):79–90
Zandalinas SI, Fritschi FB, Mittler R (2020) Signal transduction networks during stress combination. J Exp Bot 71(5):1734–1741
Zeng XH, Lin WF, Xie GS, Wang L, Cai MD, Chen JM (2007) Vigour of rubber seeds and the growth of rubber seedlings under high osmosis stress. ChinJ Trop Agri 27(2):1–5
Zhang Y, Leclercq J, Montoro P (2016) Reactive oxygen species in *Hevea brasiliensis* latex and relevance to Tapping Panel Dryness. Tree Physiol 37(2):261–269
Zhang Y, Leclercq J, Wu S, Ortega Abboud E, Pointet S, Tang C, Hu S, Montoro P (2019) Genome-wide analysis in *Hevea brasiliensis* laticifers revealed species specific post transcriptional regulations of several redox related genes. Sci Rep 9(1):5701. https://doi.org/10.1038/s41598-019-42197-8
Zeng C, Wang W, Zheng Y, Chen X, Bo W et al (2010) Conservation and divergence of microRNAs and their functions in Euphorbiaceous plants. Nucleic Acids Res 38:981–995
Zhiyi N, Guijuan K, Yu L, Longjun D, Rizhong Z (2015) Whole transcriptome survey of the putative ATP-Binding Cassette (ABC) transporter family genes in the latex producing laticifers of *Hevea brasiliensis*. PLOS ONE 10(1):e0116857. https://doi.org/10.1371/journal.pone.0116857
Zongdao H, Yanqing P (1992) Rubber cultivation under climatic stresses in China. In: Sethuraj MR, Mathew NM (eds) Natural rubber: biology, cultivation and technology. Elsevier, Amsterdam, pp 220–238

Chapter 9
Genomic Designing for Abiotic Stress Resistant Sugarcane

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Abstract Abiotic stress in crops is a relevant and persistent problem in global agriculture scenario. Crop production technologies of the past may not hold good for the future with the climate change challenge looming in the horizon and is happening right away as we read this chapter. Several crops, traditional seeds and knowledge are lost in the battle with nature yet, the ever resilient human spirit brings in new set of tools with the help of scientific interventions to feed the increasing demand from the global population. It is heartening to see that for every challenge we face, there is a bigger network of solutions from different parts of the world. We have learnt and continue to alter our agricultural practices, food habits, and energy consumption and apply sustainable efforts for saving the soil, water and other natural resources. However, there is always little we can do when it comes to nature. With this background, the abiotic stress and its effect on an important commercial, industrial and food crop, sugarcane is discussed in this chapter. Although the modern cultivars are hybrids derived from progenitor species, efforts are underway in broadening the genetic base of sugarcane with different traits obtained from a wide germplasm pool, that includes other genera as well, to meet the current demands like drought tolerance, increased biomass for industrial and pharmacological applications, biofuel and energy related applications and finally as a sugar crop of the tropics. Various abiotic stresses and their effect on the sugarcane growth and development, the status, progress and futuristic aspects of tackling them to design a better sugarcane crop with genomics tool are discussed.

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9.1 Introduction

Sugarcane is an important commercial crop grown primarily for sugar production. Almost 85% of the total global sugar production comes from sugarcane. The bagasse and molasses, byproducts of sugar industry are used in first- and second-generation biofuel production and bioplastic manufacturing worldwide. Globally sugarcane is grown in 2.78 M ha in 92 countries producing 1.9 billion tons of cane (FAOSTAT 2020). The major sugarcane growing countries are Brazil, India, Thailand and China accounting for nearly 70% of acreage and 71% of total production. The total revenue generated and the impact on farmer's livelihood aggregately contribute immensely to India's agricultural economy. It provides employment, and livelihood security to the farmers mostly settled in developing countries. Sugarcane is seen as a potential crop to meet the rising sugar and energy demand. The huge biomass produced provides many opportunities for myriad applications in various industries. As the opportunities are many so are the challenges in production and processing of sugarcane. One of the major production constraints experienced by a long duration crop like sugarcane is abiotic stress, which takes nearly 10 months for harvesting. The changing climatic conditions in the last few decades had been major challenge in agriculture globally. In India sugarcane is planted during March to May which is peak of summer and water scarcity. The heavy rains during the vegetative stage demands waterlogging tolerance in sugarcane. Reduction up to 15–45% of cane yield is observed under waterlogged condition. In the later stages, coinciding with the time of harvesting, the crop experiences severe cold in the winters during December to January. In Brazil sugarcane is cultivated year-round and varieties are developed to mitigate drought, frost and lodging resistance. The degrading soil and water conditions add to the existing stress to the crop. High salt concentration lowers the osmotic potential which causes stunted growth reducing the cane yield up to 50% (Akhtar et al. 2003; Wiedenfeld 2008). The crop is highly prone to reduced fertility in degrading soils causing nutrition deficit, a physiological stress affecting entire crop growth and development.

Mitigating abiotic stress in sugarcane and its management is devised worldwide through systematic research and development. The focus of enhancing sugar yield vis-à-vis imparting inherent resistance to biotic stress and tolerance to abiotic stress requires an integrated approach in sugarcane improvement program. Understanding various mechanisms of sugarcane biotic and abiotic stress tolerance at phenotypic, physiological, biochemical and molecular level and addressing with the conventional and modern biotechnological tools is the right approach for sugarcane improvement. Some of the strategies that are (may be) effective in sugarcane improvement are (i) marker assisted selection (MAS) for traits governed by one or few genes, thereby use of functional genomics to develop DNA based markers for selection. Mapping and tagging of genes/quantitative trait loci (QTLs) had been less effective in improvement of quantitative traits in sugarcane. However, it provided better understanding of the crop's response to various stress. (ii) Structural and functional genomics in sugarcane have led to generation of enormous data on genomic constitution and spatio-temporal expression of gene sets. Sugarcane is a unique crop which has complex genome(s)

and the economic part is the culm that accumulates high sucrose. The complexity in enhancing the economic part is two folds; the crop has to produce high biomass as well as high sucrose. The stress factor adds to the complexity of genetic networking exponentially within the crop system. (iii) Technologies to change the genetic regulation like gene silencing and use of micro-RNA. (iv) Gene editing tools to develop novel phenotype or alter the regulatory mechanism for higher yield and imparting biotic and abiotic stress tolerance. An overview of abiotic stress and the recent developments in sugarcane abiotic stress tolerance is discussed in this chapter.

9.2 Physiology of Abiotic Stresses in Sugarcane

Fertile soil and good quality water makes sugarcane thrive very well, however most of the arable land globally is drought prone, degraded or contaminated with heavy metals. A very low proportion of arable land is irrigated; the water has become hard with high concentration of Ca^{2+} and Mg^{2+} salts. The changing climatic condition has led to increase in global temperature by 1.5 °C. Sugarcane crop is semi-perineal which makes the crop vulnerable to range of abiotic stresses in one crop cycle, starting from drought/low moisture stress, high temperature, waterlogging, salinity, heavy metals and cold. These abiotic stresses are complex and are interrelated at the molecular and gene expression level. Drought and heat stress and salinity are highly interrelated that the condition aggravates causing irreparable damage in sugarcane and crops in general. Sugarcane thrives well under large amount of water however it cannot withstand prolonged exposure to saturated waterlogging stress. Optimal temperature for growth and development is essential in sugarcane, as it succumbs to high temperature, water deficit or soil salinity. Individual or combined effect of abiotic stress factors trigger yield loss, with significant effect on juice quality and sugar recovery. Sugarcane possess certain favorable morphological, physiological and biochemical adaptations to abiotic stress situations which is regulated by intricately woven molecular networks. The physiology of the crop is significantly affected under abiotic stress which can be observed as stunted growth, reduced leaf margins, low chlorophyll content, wilting, chlorosis, necrosis, leaf drying and senescence, with lethal effects under severe stress (Gandonou and Skali-Senhaji 2015; Endres et al. 2016; Phan et al. 2016; Shrivastava et al. 2017; Javed et al. 2020; Wang et al. 2020; Kaur et al. 2022). At molecular level the plant system shows similar abiotic stress responses like inducing reactive oxygen species (ROS), proline and abscisic acid, ethylene responsive factors and gene expression modified by transcription factors like bZIP, WRKY, WUS, LFY and DREB. Identifying sugarcane genotypes tolerant to various abiotic stresses and understanding the component traits imparting tolerance would help in sustaining the production of sugar and bioenergy, as efficient use of the dwindling agricultural inputs is the need of the hour in the scenario of global climate change.

The morphological indicators in sugarcane for abiotic stress are very important for screening large accessions or breeding lines in field evaluation trials. Trait specific

characterization based on morphological indicators like leaf rolling, pigmentation, chlorosis and necrosis under abiotic stress condition will aid in selection and identification of stress tolerant lines. A genotype with higher degree of stress tolerance can be used as a genetic stock in crop improvement. Other than morphological traits a narrow down approach to physiological indicators like relative water content (RWC) in leaf, root elongation, water use efficiency (WUE), photosynthetic rate (Pn), membrane stability, ionic flux, chlorophyll content and levels of proline or abscisic acid help in understanding the underlying mechanism of stress tolerance. Phytohormones have a major role in stress adaptation, wherein abscisic acid (ABA), ethylene, and cytokinins are implicated in perception, integration, and transduction of various environmental cues to alleviate abiotic stresses (Wilkinson et al. 2012). Plants exposed to moisture stress, high temperature, salinity, and cold stress respond with enhanced ABA accumulation, resulting in cellular dehydration. Based on the physiological mechanism of stress mitigation in sugarcane, in-depth studies made through genomics, transcriptomics and proteomics in model plants will unravel the complete networking of genetic regulation underlying the mechanism of stress tolerance.

9.2.1 Moisture Stress and Heat Tolerance

Sugarcane is one of the high water demanding crops, requiring 1000 to 2900 mm irrigation water, depending on the agroecological conditions (Robertson and Muchow 1997). WUE measured in Hawaii, Australia, and South Africa ranged from 4.8 to 27.0 tons cane per mega liter of water (Kingston 1994; Robertson and Muchow 1997). Since majority of water absorbed by plants is lost as transpiration, only 1–2% of the water is utilized by plants for photosynthetic and metabolic processes. Moderate moisture stress at actively growing stage of the sugarcane crop with fully developed canopy can lead to as much as 60% reduction in biomass (Robertson et al. 1999) (Fig. 9.1). Planted setts experiencing moisture stress inhibits root meristem which leads to poor establishment of sugarcane crop (Panje and Rao 1964). Soil moisture potential close to zero is the most ideal condition for sprouting of buds and at −2.0 MPa, the germination of buds completely ceases (Inman-Bamber and Smith

Fig. 9.1 Effect of moisture deficit stress on sugarcane at grand growth phase. *Source* Krishnapriya et al. (2020)

2005). Root hydraulic conductance is closely associated with leaf area expansion. Due to the strong correlation between root length and total leaf area, the latter may be used as a surrogate trait to screen for root length density (Van Antwerpen 1999).

Culm elongation is the most sensitive character to moisture stress in sugarcane (Nable et al. 1999; Koonjah et al. 2016), followed by leaf elongation, which in turn reduces photosynthetic area and total canopy Pn (Inman-Bamber and Smith 2005; Koonjah et al. 2016; Singels et al. 2010). Sugarcane in general is relatively tolerant to moisture stress, but even under moderate stress, the crop yield may be drastically reduced (Basnayake et al. 2012). Greater shoot number with stunted growth, larger length of roots with higher rate of root extension per day facilitate mining of water from deeper water tables while drought tolerance was also related to capacity of producing fresh functional roots under very low moisture conditions (Shrivastava et al. 2003). Rate of expansion in young internode declines with moisture stress along with decreasing RWC (Vasantha and Rao 2003). Thin stalked varieties with more number of millable canes, lower shoot: root ratio, deeper and extensive root system aids in maintaining higher leaf sheath moisture at 105–165 days after planting (Shrivastava et al. 2015). Simulation studies using APSIM-Sugarcane model indicated that increasing the rooting depth resulted in 20% increase in biomass accumulation, as deeper rooting was beneficial in the shallow than the deep soil which had a smaller fraction of stored water (Inman-Bamber et al. 2012).

Under water stressed conditions, root hydraulic conductance of drought-tolerant cultivars was twice that of susceptible clones (Saliendra and Meinzer 1992). Clones with higher root hydraulic conductance maintainthe isohydric condition in even under severe moisture deficit in soil. The crop maintains relatively constant leaf water potential by regulating stomatal closure (Saliendra and Meinzer 1989), coordinated by stomatal action, water status of the roots and root-derived signaling metabolites in the xylem sap (Meinzer and Grantz 1990; Meinzer et al. 1991). Leaf water potential and Pn show a strong correlation in sugarcane, wherein for every 0.1 MPa decrease in water potential, Pn decreases by 1.6 μmol m^{-2} s^{-1}. For every 1 °C rise in leaf temperature from 25 to 45 °C, Pn reduced by 0.4 μmol m^{-2} s^{-1}. With decline in the available soil moisture due to stress, water potential, osmotic potential and RWC in sugarcane varieties reduced by 46, 50 and 25%, respectively (Pooja et al. 2018). Leaf anatomical characters associated with drought tolerant sugarcane genotypes are lower frequency of stomata and stomatal index, and smaller cell sizes (Shrivastava et al. 2003). Water stress significantly reduced SPAD chlorophyll index, stomatal conductance (gs), Pn, transpiration rate (Tr) and transpiration use efficiency of photosynthesis, leading to significant reduction in shoot biomass (Zhao et al. 2013; Pooja et al. 2018). These indices may be useful for evaluation of genotypes for stress tolerance. Simulation studies indicated that increased intrinsic WUE and reduced gs leading to increased transpiration efficiency are best traits for selection of sugarcane clones in water-limited environments (Inman-Bamber et al. 2012). Traits such as gs, Tr and SPAD chlorophyll index may be given priority during breeding programs for drought tolerance and also to promote considerable gains in Pn (da Silva et al. 2012). Sugarcane clones with significant reduction in canopy temperature depression (CTD), chlorophyll fluorescence (F_v/F_m) and leaf rolling index under water-limited

condition recorded significant positive correlation with cane yield, indicating their usefulness for selecting tolerant clones (Arunkumar et al. 2020).

Under natural field condition, moisture and heat stress occur simultaneously, nevertheless temperature of 38 °C increased leaf and tiller emergence in sugarcane (Bonnett et al. 2006). In general, sugarcane is tolerant to heat stress, which is evident by some of the practices followed in sugarcane cultivation. In Australia, sugarcane setts are subjected to high temperature (52 °C) for 2 h as a part of phytosanitization. High temperature not exceeding 38 °C with two folds elevated CO_2 level resulted in significantly higher leaf area, dry matter production and juice volume (Vu et al. 2006). These studies indicate that heat tolerance can be used as a proxy trait to select drought tolerant sugarcane genotypes. In some of the studies discussed here, high temperature stress decreased leaf chlorophyll content, chlorophyll stability index (CSI), F_v/F_m, Pn, Tr, RWC and activity of enzymes such as nitrate reductase, sucrose phosphate synthase (SPS), sucrose synthase (SS), acid invertase (AI) and neutral invertase (NI) (Kohila and Gomathi 2018). On the contrary, activity of antioxidant enzymes peroxidase (POX) and superoxide dismutase (SOD) in sugarcane increased up to 15 h of exposure to 40 °C temperature stress and declined afterwards. High temperature tolerant isoforms of POX and SOD protect the cells from oxidative damage under heat stress suggesting that the plants have developed enzymatic control of scavenging the ROS under short term stress (Gomathi et al. 2013). High temperature also induced proline accumulation, total phenolics content, lipid peroxidation and soluble sugar content in all clones irrespective of their tolerance potential (Kohila and Gomathi 2018).

Elevated abscisic acid (ABA), K^+ flux and proline are biochemical indicators of response to moisture and heat stress in sugarcane. These are the major osmotic regulators in sugarcane. ABA is involved in water stress signaling and regulates stomatal conductance for maintaining water balance in sugarcane (Riera et al. 2005; Wilkinson and Davies 2010). ABA and K^+ flux has more important role in maintaining osmotic balance in sugarcane whereas, proline mostly reduces the stress-induced cellular acidification, enabling the synthesis of nucleotides and secondary metabolites to drive growth during the stress or recovery period (Hare and Cress 1997). High osmotic pressure, along with high solute concentration, less chlorophyll and carotene content, (Shrivastava et al. 2003), proline accumulation, high ratio of unsaturated fatty acids with lower membrane permeability (Shrivastava et al. 2015) are important features of drought and thermal (50–57 °C) tolerance. Peroxidase and IAA oxidase activity doubled during moisture stress, while the increase in polyphenol oxidase activity was four fold, which reverted to normal on stress recovery (Vasantha and Rao 2003). Severe water stress increased total soluble carbohydrates, proline and lipid peroxidation in sugarcane varieties (Pooja et al. 2018), whereas high temperature tolerant sugarcane genotypes exhibited higher chlorophyll content, CSI and RWC with significantly low level of lipid peroxidation and membrane injury (Kohila and Gomathi 2018).

9.2.2 Salinity Stress Tolerance

Sugarcane is a glycophyte with low tolerance to considerable high sodium ion concentration in soil. The high salt stress leads to reduction in cane yield and sucrose accumulation (Patade et al. 2011). At salinity levels of ~14 dS/m, about 50% reduction in bud sprouting and plantlet establishment was reported across a range of cultivars (Wahid and Rasul 1997; Akhtar et al. 2003). The salt tolerant genotypes similar to the moisture stress tolerant lines have shown higher Pn, gs, and shoot growth than sensitive ones at 2–12 dS/m salinity levels (Meinzer et al. 1994). Morphological traits such as pink pigmentation and waxiness in leaves, accumulation of nitrogenous solutes, restrained chlorine uptake and/or its accumulation in leaf tissue helps sugarcane to adapt to salinity stress (Shrivastava et al. 2015). Salinity reduces chlorophyll content (Winicov and Button 1991) and an overall reduction in Pn is observed (Burman et al. 2003). Juice yield and sucrose content drastically declined in sugarcane grown in salt affected soils (Lingle and Wiegand 1997).

Salt stress induces osmotic stress caused externally on plant roots and disturbs the ionic flux inside of the cell (Munns and Termaat 1986). There is surge in Na^+ ions in the cell which causes reduction in K^+/Na^+ balance in cell. Significant decrease in sugarcane biomass accumulation is observed in genotypes grown in salinity of 10 dS/m (Rao et al. 2021). High K^+ content in the juice indicates the salt-tolerant behavior of sugarcane cultivars (Lingle et al. 2000). It is observed that the K^+ ion is closely associated with moisture stress tolerance which is involved in osmoregulation of leaf water potential in sugarcane. The effect of ion toxicity is pronounced under salinity stress, as enhanced levels of leaf proline content was observed when the sugarcane plantlets were exposed to iso-osmotic stress imposed by NaCl as against mannitol (Cha-um and Kirdamanee 2009). Tolerant genotypes showed enhanced proline, polyols, and total free amino acid content than the salinity sensitive ones (Wahid 2004; Gomathi et al. 2010).

Sugarcane genotypes exhibit variation for various levels of salinity (Wahid and Rasul 1997; Vasantha et al. 2010). Progressive stress responses suggested that tolerant varieties showed stable pigment (chlorophyll and carotenoids) levels in plastids and high proline accumulation along with increased activity of oxidative enzymes. Lipid peroxidation was higher in sensitive variety, and the difference between genotypes became significant from fourth day of stress imposition (Vasantha and Rajalakshmi 2009). Gas exchange parameters were not much affected due to salinity during the formative stage, while Pn, Tr and water potential decreased significantly during grand growth of the crop. Significant reduction in sink size including cane length and stem biomass corresponded to the tolerance potential of clones (Vasantha et al. 2010). Salinity in general affected transport of sucrose from the source leaf, wherein the tolerant genotypes exhibited better sucrose biosynthesis as well as efficient partitioning towards sink tissues, reiterating that under stress, carbon allocation and partitioning was more important than the carbohydrate availability per se (Gomathi and Thandapani 2004). Salinity stress imposed at formative phase of the crop led to drastic reduction in SPAD chlorophyll index, F_v/F_m, RWC, stalk height and weight

and other yield attributes (Brindha et al. 2019). The SOS pathway genes including *SOS1* (Na^+/H^+ antiporter), *SOS2* (CIPK), and *SOS3* (CBL) associated with ion homeostasis were reported to play a major role in imparting salinity tolerance in the sugarcane variety Co 85,019. The differential accumulation of Na^+ and K^+ ions in the contrasting genotypes (Co 85,019 and Co 97,010) confirmed the role of SOS pathway in sugarcane (Brindha et al. 2021). Traits such as SPAD chlorophyll index, F_v/F_m, leaf area index and biomass production were drastically reduced under combined stresses of drought and salinity, with significant impact on the juice quality and cane yield (Vasantha et al. 2017). Some sugarcane genotypes screened in controlled salinity conditions exhibited tolerance irrespective of the phenophases and were able to maintain the leaf area at salinity level as high as 21 dS/m. It is suggested to conduct salinity screening in controlled salinity in hydroponics rather in field, owing to the high inter plot variation in soil properties in the latter (Ashraf et al. 2010). High concentration of Na^+ in soils with exchangeable sodium percent (ESP) between 3 and15 are considered sodic (Sumner and Naidu 1997). The sodicity causes poor drainage along with Ca^{2+} deficiency induced by the presence of excess Na^+ ions. Salinity also reduces the activity of nitrate reductase which hampers the uptake of nitrogen assimilation and metabolism (Mahajan et al. 2013; Medeiros et al. 2014).

9.2.3 Waterlogging and Flooding Tolerance

Intermittent flooding and waterlogging in well drained soils is not a major problem in sugarcane. However, regions with high water table up to 15 cm below the soil surface induce waterlogging stress in sugarcane. Sugarcane grown in water table ranging from 30 to 76 cm below soil surface for the entire crop cycle showed no reduction in yield up to the third ratoon (Carter and Floyd 1975). Low-lying areas with high rainfall in USA, Bangladesh, Indonesia and India experience recurring flooding and waterlogging which are one of the major production constraints to sugarcane (Bailey-Serres and Voe-senek 2008). The excess water in soil fills the voids and airspaces causing a significant reduction in O_2, CO_2, and ethylene exchange between the plant and its environment. Plants deprived of oxygen exhibit low levels of aerobic respiration and ATP production, resulting in reduced Pn and consequently plant growth. Under such hypoxic conditions, high concentration of ethylene triggers signal transduction pathways regulating various adaptive and survival responses (Bailey-Serres and Voesenek 2008). Presence of organic compounds in waterlogged soils accelerate the production of free radicals to toxic levels. Leaching and run-off of essential nutrients and secondary metabolites is common during flooding. Although sugarcane is only moderately tolerant to waterlogging stress, the water uptake patterns revealed that transpiration proceeds without much change even under flooding conditions (Chabot et al. 2002).

Flooding resulted in altered morphology including the formation of more number of adventitious roots with large aerenchyma cells Presence of aerenchymatous cells in aerial roots of tolerant cane may be a useful screening tool to identify sugarcane

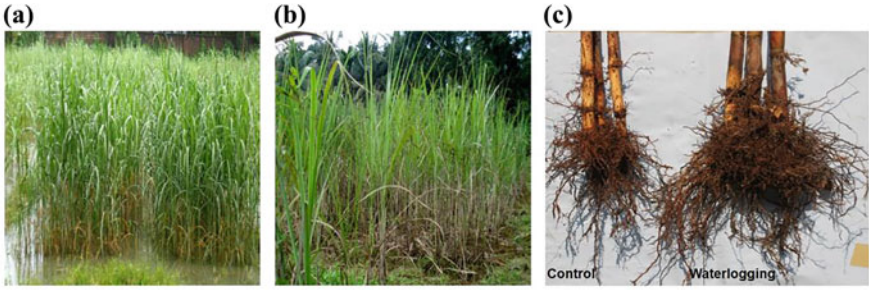

Fig. 9.2 Effect of waterlogging stress on sugarcane growth at **a** formative phase when the stress was predominant, **b** maturity phase, and **c** variation in root morphology due to waterlogging stress. Source for Fig. 9.2a, b: Gomathi et al. (2014); C: unpublished data

cultivars adapted to waterlogging stress (Gilbert et al. 2007). Flooding increased total dry weight of sugarcane, with concomitant increase in leaf, stalk and root biomass (Fig. 9.2). Sugarcane plants exposed to prolonged flooding developed three distinct roots such as reddish-black aerial roots above the water surface, whitish underwater roots from pre-existing root primordia, and thin and pinkish colored negatively geotropic roots. Root growth showed an allometric relationship, increasing along with shoot growth. Pn decreased under waterlogging, but gs and intercellular CO_2 concentration increased, indicating a non-stomatal limitation to Pn. Basal and middle internodes of the sugarcane showed higher concentration of total soluble solids measured as Brix in the flood affected plants (Tetsushi and Karim 2007).

Waterlogging stress significantly increased RWC, proline accumulation and content and activity of antioxidant enzymes SOD and POX, with considerable reduction in chlorophyll and carotenoid content (Bajpai and Chandra 2015). Traits favorable under stress included bobbin-shaped internodes, enhanced activity of polyphenol oxidase, relatively less increase in activity of alcohol dehydrogenase (ADH), aiding the quick restitution of growth upon cessation of flooding (Shrivastava et al. 2015). Leaf and stem expansion id inhibited by waterlogging stress, along with reduced tiller production and altered orientation of shoot extension. Aerial roots are important for continued supply of oxygen to the plant under flooding, in turn contributing to high dry matter production. Ethylene is also implicated in aerenchyma formation in adventitious roots under flooding stress. Genes ADH, ACC oxidase, submergence induced proteins and G-box binding factor-1 were up regulated in tolerant sugarcane varieties (Gomathi et al. 2014).

9.2.4 Cold Stress Tolerance

Sugarcane is generally cold sensitive, although the magnitude of damage depends on the severity and duration of stress, inherent resistance of cultivar, and time lapse and/or temperature fluctuations between cold stress and harvest (Tai and Lentini

1998). The wide variation in response to cold stress among sugarcane varieties was evident from field observations. wherein, sub-tropical clones were more tolerant to stress compared to their tropical counterparts (Du et al. 1999). Using the available germplasm to identify cold-responsive genes in sugarcane would greatly advance conventional breeding programs as well as development of transgenic plants with improved tolerance to cold stress. Requirement of an optimum growing temperature is one of the common factors limiting the geographical distribution and growing season of many plant species. Sugarcane cultivation, mostly restricted to tropical and sub-tropical regions does not experience drastic reduction in temperature during its crop season, although significant reduction in yield and juice quality has been observed in the event of cold stress. Cold acclimation is regulated at the post-transcriptional level in sensitive crops such as rice, corn, cotton and tomato, wherein ice formation occurs during cold stress. Several genes induced by drought stress are also expressed during cold stress, as they are commonly regulated by the phytohormone ABA. When the gene encoding the enzyme isopentenyltransferase (ipt) was overexpressed in sugarcane cv. RB855536 along with a cold inducible promoter *AtCOR15a*, the senescence rate of excised leaves subjected to low temperature reduced considerably as compared to control plants (Belintani et al. 2012). Overexpression of *ipt* gene enhanced cold tolerance of non-acclimated whole plants, with 31% higher total chlorophyll content compared to control plants, reduced malondialdehyde content, and electrolyte leakage indicating less damage due to stress. Expression of *ipt* driven by the stress inducible *COR15a* promoter enhanced tolerance to cold stress in sugarcane without negative effect on plant growth. In a similar study, the gene encoding α-tubulin from tolerant sugarcane variety GT28 (*SoTUA*)) was overexpressed in cold susceptible variety ROC22 (Chen et al. 2020). Increased expression of α-tubulin in the transgenic lines improved the soluble protein, sugars and peroxidase activity, while malondialdehyde content reduced considerably under chilling treatment as compared to control plants. Likewise, cold-defense related genes showed higher expression in the transgenic lines overexpressing *SoTUA*, indicating its protective role during chilling stress (Chen et al. 2021).

9.3 Sugarcane Genetic Resources for Abiotic Stress Tolerance

Sugarcane belongs to *Andropogoneae* tribe in the family *Poaceae*. The subtribe *Saccharine* includes the genus *Saccharum* and other related genera such as *Erianthus* (Fig. 9.3a), *Miscanthus*, *Narenga* and *Sclerostachya*. Genus *Saccharum* comprises three cultivated species *Saccharum officinarum*, *S. barberi* and *S. sinense*, and three wild species *S. robustum*, *S. spontaneum* (Fig. 9.3b) and *S. edule*. These six species of *Saccharum* along with related genera form the basic genetic resources of sugarcane, together termed as the 'Saccharum Complex' (Mukherjee 1957; Daniels et al. 1975). The entire gamut of sugarcane genetic resources is conserved in two world

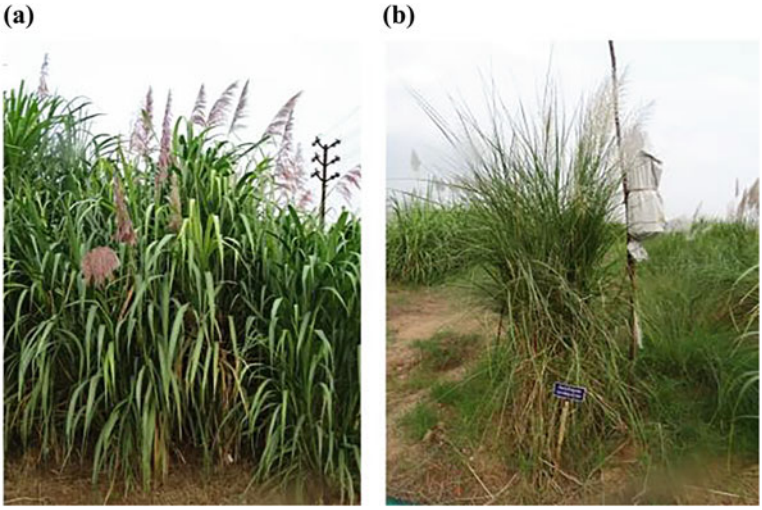

Fig. 9.3 Valuable genetic resources for abiotic stress tolerance in sugarcane (A) *Erianthus* spp. and (B) *Saccharum spontaneum* (b) clones

repositories; one in India at the ICAR-Sugarcane Breeding Institute (SBI) Regional Centre, Kannur, Kerala and the other in USA at the World Collection of Sugarcane and Related Grasses (WCSRG), Miami, Florida.

The ICAR-SBIRC repository is the world's largest in situ germplasm collection, with 2397 accessions of different *Saccharum* sp., allied genera and man-made historical and commercial hybrids (Table 9.1). Another set of germplasm consisting of 1709 *S. spontaneum*, 406 *Erianthus* sp. and 63 allied genera collected across India and exotic clones from different parts of the world are conserved in the field gene bank of ICAR-SBI Coimbatore (Table 9.1). In addition to this, 2013 clones including Co and

Table 9.1 World collection of sugarcane germplasm available at ICAR-Sugarcane Breeding Institute, India

Location	Species	Accessions
Kannur (Kerala)	*S. officinarum*	757
	S. robustum	129
	S. barberi	42
	S. sinense	30
	S. edule	16
	S. spontaneum	387
Coimbatore (Tamil Nadu)	*S. spontaneum*	1709
	Erianthus arundinaceous	230
	Erianthus sp.	176
	Allied genera	63

Co allied hybrids, interspecific and inter generic hybrids, foreign hybrids and other genetic stocks like CYM, CD clones etc. are maintained at ICAR-SBI, Coimbatore. Few *S. spontaneum* clones collected from Arunachal Pradesh, and *Erianthus* sp. and *Mischanthus sp.* clones collected from Meghalaya are maintained at Indian Agricultural Research Institute (IARI) Regional Station, Wellington, Tamil Nadu. Apart from this, a total of 1380 genotypes including Co canes, Co allied hybrids, exotic clones, inter-specific and inter-generic hybrids, core collection of *S. officinarum*, species clones of *S. barberi*, *S. sinense*, *S. robustum*, *Erianthus* sp., S*clerostachya* sp. and *Narenga* sp. are maintained at ICAR-SBI Research Centre, Agali, Kerala.

One of the important strategies for crop improvement is through wide hybridization, which is possible due to the vast germplasm collection available in sugarcane. Wild germplasm sources impart genes for high biomass producing ability, resistance to pests and diseases and adaptability for growth under different stress conditions in the modern cultivars. Nobilization of sugarcane is one of the pioneering works in the modern history, which involves planned introgression of wild forms of *Saccharum* sp. and related genera into noble canes, to improve yield and ancillary characteristics. The first interspecific hybrid released as a variety of sugarcane (Co 205) was derived from hybridization of *S. officinarum* clone Vellai and *S. spontaneum* clone Coimbatore local, leading the future course of sugarcane breeding across the globe. Modern sugarcane cultivars are complex aneupolyploids derived from the crosses involving *S. officinarum*, *S. spontaneum*, *S. barberi*, *S. sinense* and *S. robustum*. The identified sources of abiotic stress tolerance are given in Table 9.2.

9.3.1 Primary Gene Pool

The primary gene pool comprises commercial sugarcane hybrids, derived from *S. officinarum*, *S. spontaneum*, *S. barberi* and *S. sinense*, because potential of cultivars may be fixed in the first sexual generation itself.

9.3.2 Secondary Gene Pool

The cultivated species *S. officinarum*, *S. barberi* and *S. sinense* form the secondary gene pool, wherein their involvement in crop improvement programs persists for a few generations of breeding.

S. officinarum

Six drought responsive candidate genes viz., *DREB1A*, *NAC2*, *Snac1*, *SHN*1, *SIZ*1 and *PIN3* involved in ABA independent pathway of drought stress response was identified in *S. officinarum* clones. *DREB1A* gene present in Fiji B and Fiji 30 clones, induced other abiotic stress responsive genes in order to maintain the water balance during stress. *NAC2* gene which was reported in rice for cold and salt tolerance was

Table 9.2 Sugarcane genotypes identified as potential sources of different abiotic stress tolerance

Species	Genotypes	References
Moisture stress tolerant genotypes		
S. officinarum	Gungera, 57 NG 73, IJ 76–412, IJ 76–564, Caledonia Ribbon	Vasantha et al. (2017)
S. robustum	NG 77–79, 57 NG-19, NG 77–146, NG 77–23, 57 NG-27, NG 77–38, NG 77–59, NG 77–122, IJ 76–336, IJ 76–337	Vasantha et al. (2017), Priji and Hemaprabha (2014)
S. barberi	Nargori, Lalri, Manga sic, MatnaShaj, Pararia Shaj, Saretha, Pathri, Kewali, Khatuia	Vasantha et al. (2017), Priji and Hemaprabha (2014)
S. spontaneum	TS 76–216, US 56–20-1, Taiwan 96, Pamba, Ponape-1, SES 32A, IND 90–805, IND 90–796, IND 85–503, Tabongo, IND 84–351, Iritty 2, SES 168, SES 600, SES 106B, SES 515/7, SES 561, IND 90–813 and *S. spontaneum* Coimbatore	Vasantha et al. (2017), Priji and Hemaprabha (2014)
S. sinense	Mcilkrum, Reha, Lalkhadi, Kalkya, Kheli, Chukche, Uba white, Ikhri	Vasantha et al. (2017), Priji and Hemaprabha (2014)
ISH clones	ISH 9, ISH 23, ISH 41, ISH 58, ISH 100, ISH 110, ISH 118, ISH 175	Vasantha et al. (2017)
Erianthus sp.	IK76-81, IK 76–48, IK 76–62, IK 76–91, IK 76–99, IND 84–863	Augustine et al. (2015), Priji and Hemaprabha (2014)
Saccharum sp. clones	Co 997, Co 86011, Co 1148, Co89003, Co 720, Co 86032, CoLk 8102, Co 86010, ISH 100, Co 85019, Co 86002, Co 85004, Co 87023, CoC 671, Co 97008, BO 91, Co 88025, Co 2000–10, Co 99006, Co 94008, Co 98008, Co 99008, NS 83/247, Co 740, Co 419	Hemaprabha and Simon (2012)
Salinity stress tolerant genotypes		

(continued)

Table 9.2 (continued)

Species	Genotypes	References
S. officinarum	Blanche reunion, Chapina, Fiji 28, Tjiing Bali, Green german, Horne, Hawaii original 24, Hina Hina 18, IJ 76- 315, IJ 76–316, IJ 76–422, IJ 76–470, Keong, Koelz 1132, Kaludaiboothan, Luzon white, Manteiga 1295, Manteiga 1585, Manjiri red, Maxwell, Mogali, Miavoi, Mikokio- 44, Maur-55 str, Mongetgayam, NC-15, NC-33, NG 21–12, Local red, Waxy red, NG 77–67, NG 77–70, NG 77–92, NG 21–42, 21 NG-2, 21 NG-5, 21 NG-6, 21 NG-21, 28 NG 12, 28 NG 13, 28 NG 21, 28 NG 32, 28 NG 54, 28 NG 68, 28 NG 72, 28 NG 80, 28 NG 87, 28 NG 110, 28 NG 206, 28 NG 210, 28 NG 211, 28 NG 273, 28 NG 287, 51 NG 11, 51 NG 12, 51 NG 14, 51 NG 53, 51 NG 59, 51 NG 77, 51 NG 147, 51 NG 159, 51 NG 287, 57 NG 26, 57 NG 57, 57 NG 67,57 NG 68, 57 NG 100, 57 NG 126, 57 NG 71, 57 NG 159, 57 NG 166, 57 NG 172, 57 NG 184, 57 NG 191, 57 NG 196, 57 NG 198, 57 NG 199, 57 NG 203, 57 NG 237, 57 NG 241, 57 NG 272, 77 NG 15, 77 NG 18, 77 NG 31, 77 NG 32, 77 NG 65, 77 NG 66, 77 NG 117, Old Jamaica, Ogle's selection, Oramboo, Otaheite, Pynmana ribbon, Pattacheruku, Pakaweli -2, Patta Patti, Pohina -51, Selemibali, Shamsara, Sinense, Sarawak unknown, Tahiti -3, Tibbomird, UB-1, UB-14, White transparent, Zwart manila, Tjerpering, Koelz 11,132, 57 NG 78, 57 NG 215, 57 NG 50, 57 NG 212, 57 NG 110, Poona, Caledonia, Fiji 10, IM 76 - 360, IM 76- 252, Katha, Uba white, Ansali, 77 NG 242, Rayada, IJ 76 543, IJ 76 522, IJ 76 556, IJ 76 470, IJ 76 418, IJ 76 316, IJ 76 315, IM 76–507, IM 76–253, IM 76–232, Black Fiji, IK 76–31, 77 NG 1, 77 NG 221, Zwart Cheribon	Vasantha et al. (2017)
S. robustum	28 NG 219, 28 NG 251, 57 NG 6, 57 NG 201, 77 NG-10, 77 NG-26, 77 NG-34,77 NG -55, 77 NG-136, 77 NG-160, 77 NG-167, 77 NG-170, 77 NG-221, 77 NG-237, 57 NG 231	Vasantha et al. (2017)
S. barberi	Khakai, Khatuia-124, Kewali 14G, Kuswarottur, Lalri, Nargori, Pansahi, Pathri, Uba seedling, Reha	Vasantha et al. (2017)

identified from *officinarum* clones viz., 28 NG 224, Keong, 21 NG 2, Fiji B and Fiji 30. The three genes *Snac1*, *SHN1* and *SIZ1* which were reported to be involved in drought tolerance mechanism in other crops. Among these three, *Snac1* was reported to be present in 28 NG 210, SHN 1 in Penang and SIZ 1in Keong. *PIN3* gene a regulator of auxin efflux was found to be present in seven clones viz. 57 NG 136, 28 NG 224, Keong, Mia Moi, 28 NG 210, Fiji B and Fiji 30 (Priji and Hemaprabha 2014).

S. barberi

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A*, and *SIZ1* of ABA independent pathway were found to be present in *S. barberi* clones Kewali and Khatuia. Saretha clone harboured nine drought responsive genes without *DREB1A* and *Hep2*. Pathri contained all the ten genes excluding *HRD* gene (Priji and Hemaprabha 2014).

S. sinense

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A*, and *SIZ1* of ABA independent pathway were reported to be present in *S. sinense* clone Ikhri. Chuckche harbored seven drought responsive genes apart from *DREB1A*, *DRF1*, *HRD* and *SHN1*. Uba White clone was with eight drought tolerant genes without *DRF1*, *SHN1* and *Wrky 38* (Priji and Hemaprabha 2014).

9.3.3 Tertiary Gene Pool

S. spontaneum and *S. robustum* constitute the tertiary gene pool, contributing to abiotic stress tolerant traits and improvement in fiber content of the progenies. These are considered tertiary as it takes a number of generations to eliminate their undesirable effects during varietal development.

S. spontaneum

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A*, and *SIZ1* of ABA independent pathway were reported to be present in *S. spontaneum*. All these 11 genes were found to be present in three *S. spontaneum* clones viz., Iritty 2, SES 600 and *S. spontaneum* Coimbatore. Four drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2* and *Wrky 38 factor* were identified in eight clones of *S. spontaneum* viz., Iritty 2, SES 168, SES 600, SES 106B, SES 515/7, SES 561, IND 90–813 and *S. spontaneum* Coimbatore. All these eight genotypes except SES 168 harbored *Snac1* and *Hep2* genes. Similarly, *HRD*, *SHN1* and *PIN3* were found to be present in all these eight genotypes except SES 106B. SES 515/7 and SES 561 were bestowed with nine drought responsive genes excluding *PIN3* and *SHN1*. IND 90–813 harbored eight

drought responsive genes excluding *DREB1A*, *SIZ1* and *PIN3* (Priji and Hemaprabha 2014).

S. robustum

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A*, and *SIZ1* of ABA independent pathway were found to be present in *S. robustum* clone NG 77–59. The clone IJ 76 33 contained nine drought responsive candidate genes without *DREB1A* and *DRF1* genes. Similarly, nine drought responsive candidate genes excluding *SIZ1* and *Wrky 38* were present in the clones IJ 76 336 and NG 77–122 (Priji and Hemaprabha 2014).

9.3.4 Distant Gene Pool

The allied genera including *Erianthus, Mischanthus, Narenga and Sclerostachya* can be termed as distant gene pool. In this group only *E. arundinaceous* has been utilized for its high biomass and abiotic stress tolerance.

***Erianthus* species**

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A* and *SIZ1* of ABA independent pathway were reported to be present in five *Erianthus* sp clones (IK 76–48, IK 76–62, IK 76–91, IK 76–99 and IND 84–863) except *Snac1* in IK 76–91, IND 84–363 and *Wrky 38* in IK 76–99, respectively, making it an important source of drought tolerance (Priji and Hemaprabha 2014). In *E. arundinaceous* the expression of *HSP70* was found to be enhanced under moisture stress. The transgenic sugarcane overexpressing *EaHSP70* exhibited enhanced cell membrane thermostability, RWC, gas exchange parameters, chlorophyll content and photosynthetic efficiency under moisture stress. The chlorophyll retention capacity increased in these plants, with higher germination and establishment under salinity stress as compared to control plants. This demonstrates the potential of *EaHSP70* gene for genetic manipulation to induce drought and salt tolerance in sugarcane (Augustine et al. 2015). The *E. arundinaceus* clone IK 76–81 was found to be drought tolerant with increased expression of *DREB2* and expansin genes with increase in soil moisture stress (Augustine et al. 2015).

9.4 Designing Sugarcane for Abiotic Stress Tolerance

Genome organization of sugarcane reveals a complex structure. The sugarcane cultivars are in general interspecific hybrid clones of *Saccharum officinarum* ($2n = 80$ and $X = 10$) and *Saccharum spontaneum* ($2n = 40$ to 128; and also the basic chromosome number variying from $X = 4$ to 8) (D'Hont et al. 1998; Grivet and Arruda 2002). This sugarcane complex (Mukherjee 1957), show a complex ancestry resulted from

interbreeding among the six species of *Saccharum* (i.e. *S. officinarum*, *S. robustum*, *S. spontaneum*, *S. barbari*, *S. sinense*, *S. edule*) and allied genera (i.e. *Erianthus*, *Sclerostachya*, *Miscanthus*, and *Narenga*). In sugarcane breeding, modern cultivars are derivatives of *Saccharum officinarum* and *Saccharum spontaneum* interspecific crosses. *S. officinarum* is contributes thick stalk (biomass) and high sucrose whereas, biotic and abitotic stress tolerance is imparted by *S. spontaneum.* However, *S. spontaneum* is one of the progenitor of *S. officinarum* (Babu et al. 2010)*;* and the ancestry of *S. barbari* and *S. sinense* is traced back to *S. officinarum* and *S. spontaneum* (Amalraj and Balasundaram 2006). The other *Sacchrum* spp and related genera are used in the pre-pre breeding program for introgression of genes for higher biomass, resistance to pest and diseases and tolerance to abiotic stress or enhanced fitness, *Saccharum* species like *S. barberi* and *S. sinense, S. edule* and allied genera like *Erianthus*, *Sclerostachya*, *Miscanthus*, and *Narenga.* The interspecific crosses of promising sugarcane parents are planted to generate a series of hybrid clone which are subsequently selected for yield and sugar related parameters. Multi-location trials are conducted to test stability and suitability of hybrid clones in target environment. The genomic structure or organization is completely ignored, as the clones are selected by planting setts. The unavailability of chromosomal organization and the information on contribution of parents in manifestation of traits does not hamper selection or release of cultivars in sugarcane. Based on pedigree and lineage of commercial cultivars the sugarcane genome can be characterized as complex of multiple genome fragments (homoeologus), polyploid and multiple gene copies. With the available technology it is extremely difficult to ascertain which allele from which genome is expressing and also the inter- and intra – allelic interaction in the genome(s). A complete account of complexity of sugarcane genome and the challenges in genome analysis is passably reviewed by Thirugnanasambandam et al. (2018). The sugarcane improvement program relies more on chromosomal organization rather than recombination in cross derivatives. The cross derivatives show high rate of aneuploids, which masks the effects of recombination occurring in one or two generations. Under this scenario determination the breeding value of the clone is currently not achievable. The expression profiling and localization of large effect QTLs for complex traits in sugarcane are mere dissection of already formed variety. The tremendous diversity of sugarcane transcripts reveal complexity of gene expression networking in sugarcane (Thirugnanasambandam et al. 2017, 2018, 2019, 2020).

9.4.1 Conventional Breeding and Selection Procedure

Sugarcane improvement for abiotic stress tolerance is challenging. Series of intra and inter specific crosses are made to shuffle genome(s) for achieving higher yield and high sucrose content. The most important part of sugarcane breeding is the phenotyping for evaluating yield levels and stability of expression over locations and years. Multi-location evaluation of sugarcane hybrids and selection of clones for target environment is one of strategies which is practiced and followed till date.

Creating facilities for screening of sugarcane for artificial abiotic stress tolerance is highly challenging. Most of the parameters that can play significant role under natural conditions cannot be imitated in artificial conditions. The abiotic stress in sugarcane is mostly location specific and must be addressed by continuous screening in the target locations. All the scientific reports or research publications indicate that the sugarcane genotypes which performed better under near ideal condition have performed better under abiotic stress too.

9.4.2 Genomics Aided Selection in Sugarcane

In sugarcane the nature of hybrids and its progenies generated after every generation of sexual reproduction are unpredictable and hypervariable. Under such situation the prediction of performance of selfed progeny or the hybrid is very low. The low breeding value in the progenies of a hybrid discourages the use of advanced molecular tools devised for enhancing genetic gains in other crops where the chromosomal inheritance is stable, either diploid or polyploid. Some of the major challenges in practicing genomic selection are broadly classified as (i) sequencing and generation of single dose markers (ii) simplification of complexity of genome(s) in sugarcane (iii) discerning the genetic relatedness and estimating the breeding values based on the clones and parents (iv) improving the accuracy of prediction models (v) extension of one genomic selection over locations and over diverse crosses globally. Among the challenges listed above the generation of single dose marker.

9.4.3 Mi-RNA Based Selection

MicroRNAs (miRNAs) are endogenous, evolutionarily conserved RNAs which are between 19 and 24 nucleotides in length. miRNAs are master regulators of post-transcriptional phases of gene expression. They are known to interfere in translational machinery either to prevent or alter protein synthesis. When miRNA are bound to the target mRNAs, the ensuing association of decay factors lead to destabilization of mRNA (Bhaskaran and Mohan 2014). After the discovery of additional gene regulation mechanism by miRNA, researchers devised experiments to alter gene regulation in various organisms. In sugarcane and many other plant species, several studies indicated a strong role of miRNA association with abiotic stress. Involvement of plant miRNAs under stress conditions have been reported by several workers as most of their target genes are induced by stress (Jones-Rhoades and Bartel 2004; Phillips et al. 2007). Regulation of miRNA expression under stress alters the abundance of their target genes (Jagadeeswaran et al. 2009; Lv et al. 2010). Likewise, repression of miRNA causes accumulation of its target, thereby eliciting stress tolerance responses (Sunkar and Zhu 2004). Role of set of differentially expressed miRNA in sugarcane was studied with two cultivars RB867515 (drought tolerant) and

RB855536 (drought sensitive). The miRNA sp-miR394 was down-regulated under drought stress in both tolerant and sensitive sugarcane cultivars, reinstating its role in abiotic stress response. The ssp-miR394 targets the gene encoding a glyceraldehyde-3-phosphate dehydrogenase (GAPDH), while ssp-miR1432 targets bZIP in sugarcane; both these miRNAs were down-regulated under drought in both cultivars. It implies that bZIP is associated with drought response but, activation of transcription factor alone may not be responsible for differential tolerance levels in sugarcane. Under cold stress, 62 of the 412 miRNAs identified in sugarcane showed a significant differential expression (Yang et al. 2017). The cold stress induced upregulation of miR319 in roots and buds was demonstrated by subjecting sugarcane to 4 °C for 24 h (Thiebaut et al. 2012). ABA treatment is also found to trigger the miR319 production in sugarcane, with TCP-PCF5, TCP-PCF6, GAMyb, a protein kinase, and a fasciclin-like glycoprotein, a subclass of arabinogalactan proteins as potential targets. Varying periods of cold stress treatment (0–48 h at 4 °C) induced miR319, with spatial and temporal difference in expression levels in root and shoot tissues (Thiebaut et al. 2012) The up-regulation of miR319 coupled with down-regulation of its targets, a Myb transcription factor (GAMyB) and a TCP transcription factor (PCF5), were observed in cold-tolerant and -sensitive sugarcane varieties exposed to 4 °C. To narrow down the miRNAs implicated in cold tolerance, a tolerant (FN39) and sensitive (ROC22) cultivar of sugarcane was used to generate small RNA libraries, followed by validation through RT-qPCR. The miRNAs involved in targeting of auxin response factors (*ARF*) and transport inhibitor response (*TIR*) genes, miR167 and miR393 showed significant up-regulation under cold stress in both the cultivars. Differential expression of miR160 and miR156 was observed in the cultivars with contrasting cold tolerance nature in sugarcane These findings provide the valuable information for further functional characterization of miRNAs in sugarcane under cold stress. A number of environmental cues which are perceived by plants are transmitted to trigger a cascade of gene expression in response. The variation or the trigger for differential gene expression levels could be due to a number of factors starting from bio-physio-chemical mechanism like osmosis and Na^+ and Ca^{2+} flux signaling (receptors and transporters).

9.4.4 Transcriptomics of Sugarcane Abiotic Stress

Advancement in next-generation sequencing technologies have led to the development of methods for analyzing transcript abundance under various biotic and abiotic stress conditions. Nevertheless, expressed sequence tags (EST) libraries are among the earliest resources for gene discovery in several organisms including agricultural crops. EST databases facilitate large-scale mining of data mining to identify genes involved in specific pathways and traits, and hence prove to be invaluable in analyzing the global response of tissues or whole organisms under stress. A putative

model for global gene expression under cold stress was constructed using ESTs available in the Sugarcane EST Genome Project (SUCEST; http://sucest.lad.ic.unicamp.br) employing high-density filter arrays and extensive data mining (Nogueira et al. 2003). Similarly, ESTs encoding proteins directly involved in chilling tolerance identified till date include WCOR410b (Danyluk et al. 1998), WCOR413 (Allard et al. 1998), dehydrin 2 (DHN2; Zhu et al. 2000), barley ABA-inducible protein (HVA22; Shen et al. 2001), thaumatin-like protein, glucanase-like protein, and chitinase-like protein (Yu and Griffith 1999). In silico analyses confimed the presence of two putative dehydrin like proteins (WCOR410b and DHN2) in sugarcane, which aid in stabilizing macromolecules to protect cellular membranes against chilling injury (Pearce 1999). Thaumatin-like protein, glucanase-like protein (1,3-glucanase), and chitinase like protein are also implicated in pathogenesis-related response in plants, stabilizing the cellular membranes owing to their antifreeze activity, inhibiting leakage across membranes during chilling (Hincha et al. 1997; Pearce 1999; Tomczak et al. 2002). Comparative transcriptomic analysis of cold susceptible sugarcane hybrid (CP72-1210) *versus* cold tolerant *S. spontaneum* (TUS05-05) revealed more than 600 differentially expressed genes in response to cold stress (Park et al. 2015). Expression analysis of one of the differentially expressed genes, encoding a *S. spontaneum* homolog of a NOD26-like major intrinsic protein gene (SspNIP2) showed that cold treatment for 30 min was sufficient to induce SspNIP2 by ~ 2.5 fold, which persisted even up to 24 h of stress exposure. Similarly, transcriptome profiling of low temperature tolerant *S. spontaneum* clone IND 00–1037 collected from high altitude regions of Arunachal Pradesh, India, revealed that about 2583 and 3302 genes were up- and down-regulated due to stress, respectively (Dharshini et al. 2016). Cold-responsive genes such as cold-regulated (COR), dehydrins, LEA proteins, heat shock proteins (HSP), aquaporins and osmolytes play a significant role in cold acclimatization during 24 h exposure to 10 °C stress (Dharshini et al. 2016; Selvarajan et al. 2018). Root transcriptome analyses at different time intervals after stress imposition led to the detection of a total of 4425 differentially expressed transcripts (2715 upregulated and 1710 downregulated). Major genes conferring tolerance to low temperature included COR protein, osmotin, dehydrin, HAL1, chilling tolerant divergence 1 (COLD1) and HSP90, in agreement to previous studies. Further, metabolic sensors such as proline, MDA, calcium-dependent kinase, G-coupled proteins, and histidine kinase were triggered under low temperature stress in *S. spontaneum* roots, activating the signal transduction through MYB, ERF, ARF2, DREB, CAMTA, and C2H2. This resulted in the biosynthesis of annexin, which mediated the plasma membrane calcium permeability and production of cold-responsive genes. Metabolic pathways such as phenylpropanoid which stimulates flavonoid biosynthesis along with synthesis of sucrose, galactose, raffinose, and fructose are involved in triggering cold-responsive TFs.

A de novo assembly of the leaf transcriptome of two sugarcane cultivars (tolerant, SP81-3250 and susceptible, RB855453) were evaluated by the RNA-Seq method. Water deficit stress on the 90th day of stress imposition showed altered gene expression in the tolerant cultivar, while the sensitive cultivar showed differently expressed genes as early as on the 30th day of stress (Belesini et al. 2017). Several important gene families, including aquaporins, late embryogenesis abundant proteins,

auxin related proteins, transcription factors, HSPs, light harvesting chlorophyll a-b binding proteins, disease resistance proteins, and ribosomal proteins were induced in a wild sugarcane type, *S. narenga* exposed to drought stress for 22 days (Liu et al. 2018). Likewise, transcriptomic changes under varying levels of water deficit stress in tolerant hybrid (Co 06022) was compared to susceptible hybrid (Co 8021), revealing a progressive decrease in the expressed genes as the stress period increased from 6 to 10 days (Selvi et al. 2020). The *S. spontaneum* clone GXS87-16 ws considered to be a valuable resistance source to various biotic and abiotic stresses, as it was also profiled for drought responsive genes using RNA-Seq at three water-deficit levels (mild, moderate, and severe) and upon recovery during the elongation stage (Li et al. 2021).

9.4.5 Proteomics

Differentially expressed proteins under moisture and salinity stress were identified in sugarcane genotypes (Sugiharto et al. 2002; Jangpromma et al. 2010; Pacheco et al. 2013; Passamani et al. 2017). Among the many differentially expressing transcripts, ScDR1 and ScDR2 were found to play a predominant role in imparting stress tolerance to sugarcane (Begcy et al. 2012, 2019). With the advancement in screening techniques and use of recombinant DNA technology researchers could put forth the results more convincingly in sugarcane. Chen et al. (2017) reported a novel gene in sugarcane encoding a 10.66 kDa Non-specific Lipid Transfer Protein (*ScNsLTPs*), with 671 bp long cDNA, a 312 bp open reading frame (ORF). Results from RT-qPCR results showed that the overexpression of *ScNsLTPs* under stress was exogenously induced by salicylic acid, PEG and cold. However, treatment with methyl jasmonate downregulated the expression of ScNsLTPs. The genes *ScCBL2-1*, *ScCBL3-1*, and *ScCBL4* in sugarcane possessed ORF in the range of 642 to 678 bp, and encoded polypeptides containing 213 to 225 amino acids. The ScCBL protein expression in transgenic sugarcane was localized in the plasma membrane and cytoplasm. Expression of the CBL genes in *Escherichia coli* cells confirmed their role in enhancing tolerance to salinity (NaCl) and heavy metal ($CuCl_2$) stress. Resistance to invasion by *Ralstonia solanacearum* was observed in *ScCBLs* overexpressed *Nicotiana benthamiana* leaves (Su et al. 2020).

The G-protein-coupled receptors (GPCRs) were implicated in conferring tolerance to multiple abiotic stresses. The GPCRs regulate the G-protein-mediated signaling thereby influencing plant growth, development, and stress responses. The sugarcane ShGPCR1 protein sequence contained nine predicted transmembrane (TM) domains connected by four extracellular and four intracellular loops, which could interact with various ligands and heterotrimeric G proteins in the cells. Abiotic stresses including moisture deficit, salinity and low temperature unregulated the expression of *ShGPCR1*, predominantly localized to the plasma membrane. The protein ShGPCR1 helps in maintaining cell membrane integrity under stress by enhancing intracellular Ca^{2+} levels in response to GTP. Constitutive expression of

ShGPCR1 in transgenic sugarcane led to enhanced expression of genes encoding late embryogenesis abundant protein, dehydrin drought responsive 4, and galactinol synthase under moisture stress; ethylene responsive factor 3, salt overly sensitive 1, and vacuolar Na+/H+ antiporter 1 under salinity stress; and nam/ataf1/2/cuc2, cold responsive factor 2, and alcohol dehydrogenase 3 under cold stress. Transgenic events with overexpression of ShGPCR1 conferred tolerance to drought, salinity and cold stress, confirmed by estimation of RWC in the transgenic stressed plants (Ramasamy et al. 2021). Such stress tolerant transgenic lines may enhance sugarcane production in marginal environments with limited resources. The sugarcane catalase 1 (ScCAT1) protein localized in plasma membrane and cytoplasm was upregulated by smut infection as well as treatments induced by salicylic acid, methyl jasmonate, ABA, H_2O_2, heavy metal ($CuCl_2$), hyper-osmotic (PEG) and salt (NaCl) stresses (Su et al. 2014).

9.4.6 Transgenics for Abtioic Stress Tolerance

Transgenic technology in crops is a powerful tool to introduce novel traits, altering gene expression and silencing. The first transgenic sugarcane plant was developed by Bower and Birch (1992), employing an efficient microprojectile bombardment of the embryogenic callus. The methodology was further optimized for development of herbicide resistant transgenic sugarcane plants (Gallo-Meagher and Irvine 1996). Budeguer et al. (2021) published a comprehensive reviewed of genetic transformation in sugarcane. The list of drought tolerant sugarcane varieties developed though transformation is presented in Table 9.3.

Apart from the structural genes, transcription factors (TFs) may be promising candidates to develop transgenic plants with enhanced tolerance to moisture deficit, salinity and cold stress. The COR/DREB family of TFs were the first to be associated with gene regulation under abiotic stress situation (Moran et al. 1994). Enhanced drought by overexpression of DREB(s) was demonstrated by recording physiological traits like RWC, Pn, sucrose content and bud sprouting in transgenic sugarcane plants exposed to drought stress (Reis et al. 2014; Augustine et al. 2015). Constitutive expression of DEAD-box helicase gene from pea (*PDH45*) improved the salinity tolerance of sugarcane variety Co 86032. Presence of *PDH45* significantly improved cell membrane thermostability, RWC, gas exchange parameters, chlorophyll content, and photosynthetic efficiency of transgenic events of Co 86,032 under moisture stress compared to WT. Overexpression of *PDH45* also led to the upregulation of DREB2-induced downstream stress-related genes in sugarcane, resulting in higher germination ability and better chlorophyll retention compared to WT under salinity stress (Augustine et al. 2015). The role of membrane-bound receptor proteins, such as GPCRs is demonstrated to improved abiotic stress tolerance in sugarcane. GPCRs

Table 9.3 Transgenic sugarcane varieties with improved abiotic stress tolerance

Abiotic stress	Promoter	Candidate gene	Gene function	Variety used for transformation
Drought	P35S enhanced	*Tsase*	Biomolecules stabilization	ROC10
Cold	pCOR15a	*ipt*	Cytoquinin synthesis	RB855536
Drought	P35S	*AVP1*	Osmotic regulation	CP-77–400
Drought	pRab17	*DREB2A CA*	Gene regulation	RB855156
Salinity	pAIPC inducible	*P5CS*	Proline synthesis	RB855156
Drought/Salinity	pUBI	*PDH45/DREB2*	Nucleic acids metabolism, gene regulation	Co 86032
Drought/Salinity	pUBI	*HSP70*	Cellular components stabilization	Co 86032
Drought	pUBI	*BI-1*	Programmed cell death regulation	RB835089
Drought	P35S enchanced	*AVP1*	Osmotic regulation	CSSG-668
Drought	pUBI	*SoP5CS*	Proline synthesis	Guitang 21
Salinity	pUBI	*EaGly III*	Reduced oxidative damage	Co 86032
Drought	pUBI	*AtBBX29*	Gene regulation	NCo310
Drought	P35S	*TERF1*	Gene regulation	XintaitangR22
Cold	pUBI	*SoTUA*	A-tubulin synthesis	ROC22

Source Budeguer et al. (2021) and references thereof

are associated with signal perception with a major control over plant growth, development, and response to stresses. Upregulation of *ShGPCR1* through constitute overexpression enhanced tolerance to drought, salinity and cold stress (Ramasamy et al. 2021).

9.5 Genome Editing Tools and Future Prospects in Sugarcane

Genome editing (GE) is a tool for *in-vivo* modification of DNA in the genome by creating insertion, deletion or substitution within a specific sequence. It uses engineered nucleases for creating double stranded breaks in the genome. These breaks are repaired by non-homologous end joining (NHEJ) or homology recombination (HR) for creation of site directed mutations in vivo. Meganucleases (MN), Zinc-finger nucleases (ZFNs), Transcription Activator-like Effector Nucleases (TALENs) and the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Associated Nuclease 9 are the four families of engineered nucleases available at present (Mohan 2016). GE has enabled creation of novel variant alleles or altogether new phenotype. GE is a powerful tool which has many advantages over, site directed mutagenesis and transgenics. GE in agricultural crops has multiple application including but not limited to increasing yield, nutritional quality, weed protection and tolerance/resistance to biotic and abiotic stress (Ahmad et al. 2019). The first GE milestone achieved in sugarcane was using TALEN to reduce the lignin content in cell wall to improve saccharification efficiency, facilitating higher production of lingocellulosic bioethanol (Jung and Altpeter 2016). The TALEN-mediated mutants of gene encoding O-methyltransferase showed a significant reduction in total lignin and altered lignin composition, along with 43.8% improved saccharification efficiency (Kannan et al. 2018). The complex sugarcane genome which is large, highly polyploid and aneuploid in nature poses many challenges. Targeting and localizing specific sequence in multiple genomes of sugarcane with multiple alleles and high copy are the obvious impediments in the use of genome editing tools in sugarcane. Gene silencing of the non-targeted alleles or copies in the genome can be attempted to achieve expression of single copy.

As the chapter discusses the various stresses and sugarcane's response to them, it is clearly evident that the abiotic stress tolerance forms a very large, complex, over-lapping network of several genes and transcription factors with many layers of regulation at protein, mRNA, miRNA, transcripts/alternative transcripts/transcript variants and finally the genes and genomes. In addition to these, there are various retrotransposons, transposable elements and several uncharacterized genes involved in the regulatory network of abiotic stress response and tolerance in sugarcane. The complex sugarcane genome with 12–15 copies of a gene in its large, mixed genomic composition of 2–3 progenitor genomes offers a real challenge to the present day biotechnological tools. Every genotype/variety differs in the chromosome number and composition although the abiotic stress response seemingly involves a definitive pattern as seen from the recent genomics studies. Genomic designing of sugarcane combining the best of traits, and biotic and abiotic resistance sourcing genes from different germplasms is currently a dream to plant breeders and molecular biologists with more emphasis on ever- changing, hostile environs of the global climate scenario. With more advances in genomics and computing facilities, the dream must be realized in the near future.

References

Ahmad N, Rahman M, Mukhtar Z, Zafar Y, Zhang B (2019) A critical look on CRISPR-based genome editing in plants. J Cellular Physiol 235(2):666–682

Akhtar S, Wahid A, Rasul E (2003) Emergence, growth and nutrient composition of sugarcane sprouts under NaCl salinity. Biol Plant 46(1):113–116. https://doi.org/10.1023/A:1022326604192

Allard F, Houde M, Kröl M, Ivanov A, Huner NP, Sarhan F (1998) Betaine improves freezing tolerance in wheat. Plant Cell Physiol 39(11):1194–1202

Amalraj VA, Balasundaram N (2006) On the taxonomy of the members of '*Saccharum* complex'. Genet Resour Crop Evol 53:35–41. https://doi.org/10.1007/s10722-004-0581-1

Arunkumar R, Vasantha S, Tayade AS, Anusha S, Geetha P, Hemaprabha G (2020) Physiological efficiency of sugarcane clones under water-limited conditions. Trans ASABE 63(1):133–140

Ashraf M, Ahmad R, Bhatti AS, Afzal M, Sarwar A, Maqsood MA, Kanwal S (2010) Amelioration of salt stress in sugarcane (*Saccharum officinarum* L.) by supplying potassium and silicon in hydroponics. Pedosphere 20(2):153–162

Augustine SM, Narayan JA, Syamaladevi DP, Appunu C, Chakravarthi M, Ravichandran V, Tuteja N, Subramonian N (2015) Introduction of pea DNA helicase 45 into sugarcane (*Saccharum* spp. hybrid) enhances cell membrane thermostability and upregulation of stress-responsive genes leads to abiotic stress tolerance. Mol Biotechnol 57(5):475–488

Babu C, Koodalingam K, Natarajan U, Shanthi R, Govindaraj P (2010) Genetic enhancement of sugarcane (*Saccharum* sp. hybrids) for resistance to red rot disease and economic traits. J. Agric Sci 4:97–107. https://doi.org/10.4038/jas.v4i3.1648

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

Bajpai S, Chandra R (2015) Effect of waterlogging stress on growth characteristics and SOD gene expression in sugarcane. Intl J Sci Res Pub 5(1):1–8

Basnayake J, Jackson PA, Inman-Bamber NG, Lakshmanan P (2012) Sugarcane for water-limited environments. Genetic variation in cane yield and sugar content in response to water stress. J Exp Bot 63(16):6023–6033

Begcy K, Mariano ED, Gentile A, Lembke CG, Zingaretti SM, Souza GM, Menossi M (2012) A novel stress-induced sugarcane gene confers tolerance to drought, salt and oxidative stress in transgenic tobacco plants. PLoS One 7(9)

Begcy K, Mariano ED, Lembke CG, Zingaretti SM, Souza GM, Araújo P, Menossi M (2019) Over-expression of an evolutionarily conserved drought-responsive sugarcane gene enhances salinity and drought resilience. Ann Bot 124(4):691–700

Belesini AA, Carvalho FMS, Telles BR, de Castro GM, Giachetto PF, Vantini JS, Carlin SD, Cazetta JO, Pinheiro DG, Ferro MIT (2017) De novo transcriptome assembly of sugarcane leaves submitted to prolonged water-deficit stress. Genet Mol Res 16(2):28549198

Belintani NG, Guerzoni JTS, Moreira RMP, Vieira LGE (2012) Improving low-temperature tolerance in sugarcane by expression the *ipt* gene under a cold inducible promoter. Biol Plant 56:71–77

Bhaskaran M, Mohan M (2014) MicroRNAs: history, biogenesis, and their evolving role in animal development and disease. Vet Pathol 51(4):759–774

Bonnett GD, Hewitt ML, Glassop D (2006) Effects of high temperature on the growth and composition of sugarcane internodes. Aust J Agric Res 57(10):1087–1095

Bower R, Birch RG (1992) Transgenic sugarcane plants via microprojectile bombardment. Plant J 2(3):409–416

Brindha C, Vasantha S, Arunkumar R (2019) The response of sugarcane genotypes subjected to salinity stress at different growth phases. J Plant Stress Physiol 5:28–33

Brindha C, Vasantha S, Raja AK, Tayade AS (2021) Characterization of the salt overly sensitive pathway genes in sugarcane under salinity stress. Physiol Planta 171(4):677–687

Budeguer F, Ramón E, Perera MF, Racedo J, Castagnaro AP, Noguera AS, Bjorn W (2021) Genetic transformation of rice: current status and future prospects. Front Plant Sci 12(2)
Burman U, Garg BK, Kathju S (2003) Influence of kinetin on photosynthesis, nitrogen metabolism and yield of clusterbean under moisture deficit condition. Indian J Plant Physiol 8(3):287–291
Carter CE, Floyd JM (1975) Inhibition of sugarcane yields by high water table during dormant season. In: Proceedings-American society of sugar cane technologists (USA)
Chabot R, Bouarfa S, Zimmer D et al (2002) Sugarcane transpiration with shallow water-table: sap flow measurements and modelling. Agric Water Manag 54:17–36
Cha-um S, Kirdamanee C (2009) Proline accumulation, photosynthetic abilities and growth characters of sugarcane (*Saccharum officinarum* L.) plantlets in response to iso-osmotic salt and water deficit stress. Agric Sci China 8:51–58
Chen J, Zhang J, Hu J, Xiong W, Du C, Lu M (2017) Integrated regulatory network reveals the early salt tolerance mechanism of Populus euphratica. Sci Rep 7:1–13
Chen J, Khan Q, Wei J, Tang L, Dong D, Li Y (2020) Analysis of physio-biochemical characteristics of T2 α-tubulin SoTUA transgenic sugarcane. Chin J Trop Crops 41(4):685
Chen J, Khan Q, Sun B, Tang L, Yang L, Zhang B et al (2021) Overexpression of sugarcane *SoTUA* gene enhances cold tolerance in transgenic sugarcane. Agron J 20:agj2.20618
Daniels J, Smith P, Paton N, Williams CA (1975) The origin of the genus *Saccharum*. Sugarcane Breed News 36:24–39
Danyluk J, Perron A, Houde M, Limin A, Fowler B, Benhamou N, Sarhan F (1998) Accumulation of an acidic dehydrin in the vicinity of the plasma membrane during cold acclimation of wheat. Plant Cell 10:623–638
da Silva PP, Soares L, da Costa JG, Viana LS, Andrade JCF, Goncalves EF et al (2012) Path analysis for selection of drought tolerant sugarcane genotypes through physiological components. Ind Crops Prod 37:11–19
Dharshini S, Chakravarthi M, Ashwin Narayan J, Manoj VM, Naveenarani M, Kumar R, Meena M, Ram B, Appunu C (2016) De novo sequencing and transcriptome analysis of a low temperature tolerant *Saccharum spontaneum* clone IND 00-1037. J Biotechnol 231:280–294
D'Hont A, Ison D, Alix K, Roux C, Glaszmann JC (1998) Determination of basic chromosome numbers in the genus *Saccharum* by physical mapping of ribosomal RNA genes. Genome 41(2):221–225
Du YC, Nose A, Wasano K (1999) Effects of chilling temperature on photosynthetic rates, photosynthetic enzyme activities and metabolite levels in leaves of three sugarcane species. Plant Cell Environ 22:317–324
EndresL ONG, Ferreira VM, Silva JV, Barbosa GVS, Junior SOM (2016) Morphological and physiological response of sugarcane under abiotic stress to neonicotinoid insecticides. Theor Exp Plant Physiol 28(4):347–355
FAOSTAT (2020) https://www.fao.org/faostat/en/#data/QCL. Accessed 11 Jan 2022
Gallo-Meagher M, Irvine JE (1996) Herbicide resistant transgenic sugarcane plants containing the bar gene. Crop Sci 36(5):1367–1374
Gandonou CB, Skali-Senhaji N (2015) Sugarcane (*Saccharum* sp.) salt tolerance at various developmental levels. In: Chakraborty U, Chakraborty B (eds) Abiotic stresses in crop plants. CABI eBooks, pp 102–111
Gilbert RA, Rainbolt CR, Morris DR, Bennett AC (2007) Morphological responses of sugarcane to long-term flooding. Agronomy 99:1622–1628
Gomathi R, Thandapani PV (2004) Sugar metabolism and carbon partitioning of sugarcane genotypes under salinity stress condition. Sugar Tech 6(3):151–158
Gomathi R, Vasantha S, Thandapani V (2010) Mechanism of osmo regulation in response to salinity stress in sugarcane. Sugar Tech 12(3):305–311
Gomathi R, Shiyamala S, Vasantha S, Johnson DE, Janani PK (2013) Kinetics of metabolism in sugarcane (*Saccharum officinarum* L.) under heat stress. Indian J Plant Physiol 18(1):41–47
Gomathi R, Gururaja Rao PN, Chandran K, Selvi A (2014) Adaptive responses of sugarcane to waterlogging stress: an overview. Sugar Tech 17:325–338

Grivet L, Arruda P (2002) Sugarcane genomics: depicting the complex genome of an important tropical crop. Curr Opin Plant Biol 5(2):122–127
Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul 21(2):79–102
Hemaprabha G, Simon S (2012) Genetic diversity and selection among drought tolerant genotypes of sugarcane using microsatellite markers. Sugar Tech. http://doi.org/10.1007/s12355-012-0164-y
Hincha DK, Meins Jr F, Schmitt JM (1997) B-1,3-Glucanase is cryoprotective in vitro and is accumulated in leaves during cold acclimation. Plant Physiol 114:1077–1083
Inman-Bamber NG, Smith DM (2005) Water relations in sugarcane and response to water deficits. Field Crops Res 92(2–3):185–202
Inman-Bamber NG, Lakshmanan P, Park S (2012) Sugarcane for water-limited environments: theoretical assessment of suitable traits. Field Crops Res 134:95–104
Jagadeeswaran G, Zheng Y, Li YF, Shukla LI, Matts J, Hoyt P, Sunkar R et al (2009) Cloning and characterization of small RNAs from Medicago truncatula reveals four novel legume-specific microRNA families. New Phytol 184(1):85–98
Jangpromma N, Kitthaisong S, Lomthaisong K, Daduang S, Jaisil P, Thammasirirak S (2010) A proteomics analysis of drought stress-responsive proteins as biomarker for drought-tolerant sugarcane cultivars. Am J Biochem Biotechnol 6:89–102
Javed T, Shabbir R, Ali A, Afzal I, Zaheer U, Gao SJ (2020) Transcription factors. In plant stress responses: challenges and potential for sugarcane improvement. Plants 9(4):1–18
Jones-Rhoades MW, Bartel DP (2004) Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. Mol Cell 14(6):787–799
Jung JH, Altpeter F (2016) TALEN mediated targeted mutagenesis of the caffeic acid O-methyltransferase in highly polyploid sugarcane improves cell wall composition for production of bioethanol. Plant Mol Biol 92(1):131–142
Kannan B, Jung JH, Moxley GW, Lee SM, Altpeter F (2018) TALEN-mediated targeted mutagenesis of more than 100 *COMT* copies/alleles in highly polyploid sugarcane improves saccharification efficiency without compromising biomass yield. Plant Biotechnol J 16(4):856–866
Kaur L, Meena MR, Lenka SK, Appunu C, Kumar R, Kulshreshtha N (2021) Molecular approaches for improving abiotic stress tolerance in sugarcane. In: Shanker AK, Shanker C, Anand A, Maheshwari M (eds) Climate change and crop stress: vmolecules to ecosystems. Academic Press, Cambridge, Massachusetts, pp 465–492
Kaur L, Meena MR, Lenka SK, Appunu C, Kumar R, Kulshreshtha N (2022) Molecular approaches for improving abiotic stress tolerance in sugarcane. INC
Kingston G (1994) Benchmarking yield of sugarcane from estimates of water use. Proc Aust Soc Sug Cane Technol 16:201–209
Krishnapriya V, Arun Kumar R, Gomathi R, Vasantha S (2020) Ganne kee utpaadakata par jalavaayu parivartan aur ajaivik tanaav ka prabhaav (in Hindi). Ganna Prakash Takniki Bulletin 5(5):13–17
Kohila S, Gomathi R (2018) Adaptive physiological and biochemical response of sugarcane genotypes to high-temperature stress. Indian J Plant Physiol 23(2):245–260
Koonjah SS, Badaloo MGH, Mangar M, Beekharry A, Dookun-Saumtally A (2016) A new and reliable criterion for the identification of early ripening, high-sucrose parent varieties. In: Proceedings of the international society of sugar cane technologists, vol 29, pp 893–900
Li C, Wang Z, Nong Q, Lin L, Xie J, Mo Z, Huang X, Song X, Malviya MK, Solanki MK, Li Y (2021) Physiological changes and transcriptome profiling in *Saccharum spontaneum* L. leaf under water stress and re-watering conditions. Sci Rep 11(1):5525
Lingle SE, Wiegand CL (1997) Soil salinity and sugarcane juice quality. Field Crops Res 54(2–3):259–268
Liu X, Zhang R, Ou H, Gui Y, Wei J, Zhou H, Tan H, Li Y (2018) Comprehensive transcriptome analysis reveals genes in response to water deficit in the leaves of *Saccharum narenga* (Nees ex Steud.) hack. BMC Plant Biol 18(1):250
Lv D-K, Bai X, Li Y, Ding X-D, Ge Y, Cai H, Ji W, Wu N, Zhu Y-M (2010) Profiling of cold-stress-responsive miRNAs in rice by microarrays. Gene 459(1–2):39–47

Mahajan ST, Naik RM, Dalvi US (2013) Assessment of biochemical markers in differentiating sugarcane genotypes for salt tolerance. Sugar Tech 15(2):116–121
Medeiros CD, Ferreira Neto JRC, Oliveira MT, Rivas R, Pandolfi V, Kido EA, Baldani J, Santos MG (2014) Photosynthesis, antioxidant activities and transcriptional responses in two sugarcane (*Saccharum officinarum* L.) cultivars under salt stress. Acta Physiol Plant 36(2):447–459
Meinzer FC, Grantz DA (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. Plant Cell Environ 13(4):383–388
Meinzer FC, Grantz DA, Smit B (1991) Root signals mediate coordination of stomatal and hydraulic conductance in growing sugarcane. Funct Plant Biol 18(4):329–338
Meinzer FC, Plaut Z, Saliendra NZ (1994) Carbon isotope discrimination, gas exchange, and growth of sugarcane cultivars under salinity. Plant Physiol 104(2):521–526
Mohan C (2016) Genome editing in sugarcane: challenges ahead. Front Plant Sci 7:1542
Moran JF, Becana M, Iturbe-Ormaetxe I, Frechilla S, Klucas RV, Aparicio-Tejo P (1994) Drought induces oxidative stress in pea plants. Planta 194:346–352
Mukherjee SK (1957) Origin and distribution of *Saccharum.* Bot Gaz 119:55–61
Munns R, Termaat A (1986) Whole-plant responses to salinity. Funct Plant Biol 13(1):143–160
Nable RO, Robertson MJ, Berthelsen S (1999) Response of shoot growth and transpiration to soil drying in sugarcane. Plant Soil 207(1):59–65
Nogueira FTS, De Rosa Jr VE, Menossi M, Ulian EC, Arruda P (2003) RNA expression profiles and data mining of sugarcane response to low temperature. Plant Physiol 132(4):1811–1824
Pacheco CM, Pestana-Calsa MC, Gozzo FC, Nogueira RJMC, Menossi M, Junior TC (2013) Differentially delayed root proteome responses to salt stress in sugar cane varieties. J Proteome Res 12(12):5681–5695
Panje RR, Raja Rao T (1964) Studies on the germination and moisture relationships of sugarcane setts. New Phytol 63(2):140–152
Park JW, Benatti TR, Marconi T, Yu Q, Solis-Gracia N, Mora V, da Silva JA (2015) Cold responsive gene expression profiling of sugarcane and *Saccharum spontaneum* with functional analysis of a cold inducible *Saccharum* homolog of NOD26-like intrinsic protein to salt and water stress. PLoS ONE 10(5):e0125810
Passamani LZ, Barbosa RR, Reis RS, Heringer AS, Rangel PL et al (2017) Salt stress induces changes in the proteomic profile of micropropagated sugarcane shoots. PLoS ONE 12(4):e0176076
Patade VY, Bhargava S, Suprasanna P (2011) Salt and drought tolerance of sugarcane under iso-osmotic salt and water stress: growth, osmolytes accumulation, and antioxidant defense. J Plant Interact 6(4):275–282
Pearce RS (1999) Molecular analysis of acclimation to cold. Plant Growth Regul 29:47–76
Phan TT, Sun B, Niu JQ, Tan QL, Li J, Yang LT, Li YR (2016) Overexpression of sugarcane gene *SoSnRK2.*1 confers drought tolerance in transgenic tobacco. Plant Cell Rep 35(9):1891–1905
Phillips JR, Dalmay T, Bartels D (2007) The role of small RNAs in abiotic stress. FEBS Lett 581(19):3592–3597
Priji PJ, Hemaprabha G (2014) Sugarcane-specific drought responsive candidate genes belonging to ABA-independent pathway identified from tolerant and susceptible clones of *Saccharum* and *Erianthus* species. Indian J Genet 74(1):64–72
Pooja NAS, Chand M, Singh K, Mishra AK, Kumar A, Kumari A, Rani B (2018) Varietal variation in physiological and biochemical attributes of sugarcane varieties under different soil moisture regimes. Indian J Exp Biol 57:721–732
Ramasamy M, Damaj MB, Vargas-Bautista C, Mora V, Liu J, Padilla CS, Irigoyen S, Saini T, Sahoo N, JDaSilva JA, Mandadi KK (2021) A sugarcane G-protein-coupled receptor, ShGPCR1, confers tolerance to multiple abiotic stresses. Front Plant Sci 12:745891
Rao VP, Sengar RS, Singh RB (2021) Identification of salt tolerant sugarcane cultivars through phenotypic, physiological and biochemical studies under abiotic stress. Plant Physiol Rep 26(2):256–283

Reis RR, da Cunha BADB, Martins PK, Martins MTB, Alekcevetch JC, Chalfun-Júnior A, Andrade AC, Ribeiro AP, Qin F, Mizoi J, Yamaguchi-Shinozaki K (2014). Induced over-expression of AtDREB2A CA improves drought tolerance in sugarcane. Plant Sci 221:59–68

Riera M, Valon C, Fenzi F, Giraudat J, Leung J (2005) The genetics of adaptive responses to drought stress: abscisic acid-dependent and abscisic acid-independent signalling components. Physiol Plant 123(2):111–119

Robertson MJ, Muchow RC (1997) Variation in the effectiveness of rainfall meeting crop water requirements in the Australian Sugar Industry. Proc Aust Soc Sug Cane Technol 19:229–236

Robertson MJ, Inman-Bamber NG, Muchow RC, Wood AW (1999) Physiology and productivity of sugarcane with early and mid-season water deficit. Field Crops Res 64(3):211–227

Saliendra NZ, Meinzer FC (1989) Relationship between root/soil hydraulic properties and stomatal behaviour in sugarcane. Funct Plant Biol 16(3):241–250

Saliendra NZ, Meinzer FC (1992) Genotypic, developmental and drought-induced differences in root hydraulic conductance of contrasting sugarcane cultivars. J Exper Bot 43(9):1209–1217

Selvarajan D, Mohan C, Dhandapani V et al (2018) Differential gene expression profiling through transcriptome approach of *Saccharum spontaneum* L. Underlow temperature stress reveals genes potentially involved in cold acclimation. 3 Biotech 8:195

Shen Q, Chen C-N, Brands A, Pan S-M, Tuan-Hua DH (2001) The stress-and abscisic acid-induced barley gene HVA22: developmental regulation and homologues in diverse organisms. Plant Mol Biol 45(3):327–340

Shrivastava AK, Pathak AD, Misra V, Srivastava S, Swapna M, Shukla SP (2017) Sugarcane crop: its tolerance towards abiotic stresses. In: Minhas, Singh P, Rane J, Pasala RK (eds) Abiotic stress management for resilient agriculture. Springer Nature, Singapore, pp 375–397

Shrivastava AK, Solomon S, Rai RK, Singh P, Chandra A, Jain R, Shukla SP (2015) Physiological interventions for enhancing sugarcane and sugar productivity. Sugar Tech 17(3):215–226

Shrivastava AK, Srivastava DC, Solomon S, Srivastava MK, Singh I (2003) Physiological characters imparting resistance to biotic and abiotic stresses in sugarcane. Sugar Tech 5(3):105–120

Singels A, Van Den Berg M, Smit MA, Jones MR, van Antwerpen R (2010) Modelling water uptake, growth and sucrose accumulation of sugarcane subjected to water stress. Field Crops Res 117(1):59–69

Selvi A, Devi K, Manimekalai R, Prathima PT (2020) Comparative analysis of drought-responsive transcriptomes of sugarcane genotypes with differential tolerance to drought. 3 Biotechnology 10(6):236

Su Y, Guo J, Ling H, Chen S, Wang S, Xu L, Allan AC, Que Y (2014) Isolation of a novel peroxisomal catalase gene from sugarcane, which is responsive to biotic and abiotic stresses. PLoS One 9(1): e84426

Su W, Huang L, Ling H, Mao H, Huang N, Su Y, Ren Y, Wang D, Xu L, Muhammad K, Que Y (2020) Sugarcane calcineurin B-like (CBL) genes play important but versatile roles in regulation of responses to biotic and abiotic stresses. Sci Rep 10(1):1–13

Sugiharto B, Ermawati N, Mori H, Aoki K, Yonekura-Sakakibara K, Yamaya T, Sugiyama T, Sakakibara H (2002) Identification and characterization of a gene encoding drought-inducible protein localizing in the bundle sheath cell of sugarcane. Plant Cell Physiol 43(3):350–354

Sumner ME, Naidu R (1997) Sodic soils. Oxford University Press, New York

Sunkar R, Zhu J-K (2004) Novel and stress-regulated microRNAs and other small RNAs from Arabidopsis. Plant Cell 16(8):2001–2019

Tai PYP, Lentini RS (1998) Freeze damage of Florida sugarcane. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS

Tetsushi H, Karim MA (2007) Flooding tolerance of sugarcane in relation to growth, physiology and root structure. South Pac Stud 28(1):9–21

Thiebaut F, Grativol C, Carnavale-Bottino M, Rojas CA, Tanurdzic LOS, Farinelli L, Martienssen RA, Hemerly AS, Ferreira PCG (2012) Computational identification and analysis of novel sugarcane micrornas. BMC Genomics 13:290. http://doi.org/10.1186/1471-2164-13-290

Thirugnanasambandam PP, Hoang NV, Henry RJ (2018) The challenge of analyzing the sugarcane genome. Front Plant Sci 9:1–18
Thirugnanasambandam PP, Hoang NV, Furtado A, Botha FC, Henry RJ (2017) Association of variation in the sugarcane transcriptome with sugar content. BMC Genom 18:909
Thirugnanasambandam PP, Mason PJ, Hoang NV, Furtado A, Botha FC, Henry RJ (2019) Analysis of the diversity and tissue specificity of sucrose synthase genes in the long read transcriptome of sugarcane. BMC Plant Biol 19:160
Thirugnanasambandam PP, Kasirajan L, Furtado A, Botha FC, Henry RJ (2020) Control of sugar and fibre: Insights from sugarcane transcriptome analyses. Proceedings 36:204
Tomczak MM, Hincha DK, Estrada SD, Wolkers WF, Crowe LM, Feeney RE, Tablin F, Crowe JH (2002) A mechanism for stabilization of membranes at low temperatures by an antifreeze protein. Biophys J 82:874–881
Van Antwerpen R (1999) Sugarcane root growth and relationships to above-ground biomass. In: Proceedings of the South African sugar technologists association, vol 73, pp 89–95
Vasantha S, Gomathi R, Brindha C (2017) Growth and nutrient composition of sugarcane genotypes subjected to salinity and drought stresses. Commun Soil Sci Plant Analysis 48(9):989–998
Vasantha S, Rajalakshmi R (2009) Progressive changes in biochemical characters of sugarcane genotypes under salinity stress. Indian J Plant Physiol 14(1):34–38
Vasantha S, Rao PNG (2003) Influence of moisture stress on the activity of oxidative enzymes in sugarcane. Indian J Plant Physiol 8(4):405–407
Vasantha S, Venkataramana S, Gururaja Rao PN, Gomathi R (2010) Long term salinity effect on growth, photosynthesis and osmotic characteristics in sugarcane. Sugar Tech 12(1):5–8
Vu JCV, Allen LH, Gesch RW (2006) Up-regulation of photosynthesis and sucrose metabolism enzymes in young expanding leaves of sugarcane under elevated growth CO2. Plant Sci 171:123–131
Wahid A (2004) Analysis of toxic and osmotic effects of sodium chloride on leaf growth and economic yield of sugarcane. Bot Bull Acad Sin 45:133–141
Wahid A, Rasul E (1997) Identification of salt tolerance traits in sugarcane lines. Field Crop Res 54(1):9–17
Wang D, Wang L, Su W, Ren Y, You C, Zhang C, Que Y, Su Y (2020) A class III WRKY transcription factor in sugarcane was involved in biotic and abiotic stress responses. Sci Rep 10(1):1–15
Wiedenfeld B (2008) Effects of irrigation water salinity and electrostatic water treatment for sugarcane production. Agric Water Manage 95(1):8588. https://doi.org/10.1016/J.AGWAT.2007.10.004
Wilkinson S, Davies WJ (2010) Drought, ozone, ABA and ethylene: new insights from cell to plant community. Plant Cell Environ 33:510–525
Winicov I, Button JD (1991) Accumulation of photosynthesis gene transcripts in response to sodium chloride by salt-tolerant alfalfa cells. Planta 183(4):478–483
Yang Y, Zhang X, Su Y et al (2017) Mirna alteration is an important mechanism in sugarcane response to low-temperature environment. BMC Genomics 18:833
Yu XM, Griffith M (1999) Antifreeze proteins in winter rye leaves form oligomeric complexes. Plant Physiol 119:1361–1370
Zhao D, Glaz B, Comstock JC (2013) Sugarcane leaf photosynthesis and growth characters during development of water-deficit stress. Crop Sci 53:1066–1075
Zhu B, Choi D-W, Fenton R, Close TJ (2000) Expression of the barley dehydrin multigene family and the development of freezing tolerance. Mol Gen Genet MGG 264(1):145–153

Chapter 10
Abiotic Stress Resistance in Tobacco: Advances and Strategies

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Abstract Tobacco is a commercial crop cultivated globally in about 3.62 million hectares mainly in semi-arid and rain-fed areas. The crop is often confronted with various forms of abiotic stresses viz., drought, flood, high temperature (heat), cold, salinity, ozone, low and high light intensity, chlorides, heavy metals, ultraviolet radiation etc. Further, climate change in terms of higher temperature, and changing rainfall patterns is going to have remarkable effect on tobacco productivity and quality. The abiotic stresses usually play a negative role in the growth and development of tobacco plant. Tobacco plant respond to stresses in various ways with alterations at morphological, molecular, physiological and cellular levels involving switching on or off stress responsive genes. Inherent capacity of tobacco genotypes need to be improved for achieving higher and stable yields under different abiotic stresses. Degree of abiotic stress and crop stage of stress occurrence vary from year to year and place to place making it difficult to breed resistant varieties. Currently, very fewer number of abiotic stress resistant tobacco varieties are developed through conventional breeding. The Complicated nature of abiotic stresses, lack of suitable morphology based screening techniques, polygenic nature of resistance mechanisms, lack of sources of resistance etc. are limiting the progress that could be made in abiotic stress breeding. These limitations can be successfully overcome through molecular breeding and genome designing strategies. Hence, in this chapter, an attempt made to summarize the available knowledge about tobacco genetic resources, germplasm

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characterization through molecular markers, molecular maps, markers and QTLs linked to various abiotic stresses, omics resources and databases, abiotic resistant genes studied, etc. for their utilization in designing tobacco genomes for abiotic stress resistance. Further, recent technological advances in marker-assisted breeding, gene editing and genome designing technologies were discussed for effectively utilizing them in developing abiotic stress resistant tobacco genotypes.

Keywords Tobacco · *Nicotiana* · Abiotic stress · Molecular · Markers · Maps · Genome sequencing · Linked markers · QTL · Genome designing · MAS · Gene editing · Cloning · Databases

10.1 Introduction

10.1.1 Economic Importance of the Crop

Tobacco belongs to family Solanaceae with more than 75 species, among which *Nicotiana tabacum* and *N. rustica* are the cultivated species (Goodspeed 1954; Chase et al. 2003; Sierro et al. 2014). Tobacco is a most important non-food crops cultivated in more than 120 countries (FAO 2019). Tobacco is defined by different criteria such as region of production, intended use (i.e. cigar filler, binder and wrapper, bidi, chewing, hookah and cigarette manufacturing), methods of curing (flue-, air-, sun-, smoke-, pit- and fire-cured tobacco) as well as morphological and biochemical characteristics (i.e., aromatic fire-cured, bright leaf tobacco, Burley tobacco, Turkish or oriental tobacco, etc. (Ren and Timko 2001). It is grown on wide variety of soils and climate in less than one percent of the world's agricultural land. Currently, tobacco is being grown in an area of 3.62 million hectares with the global tobacco production of 6.69 million tons (FAO 2019). Some major countries growing tobacco are China, India, Brazil, United Republic of Tanzania, Indonesia, Zimbabwe, Malawi, USA, Zambia, Mozambique, Turkey, Democratic People's Republic of Korea, Bangladesh, Argentina and Pakistan. China is the largest producer of tobacco (2.61 million tons/year) in the world followed by India (0.8 million tons/year), both accounting for more than 50% of World's total. Harvested tobacco leaves are cured and used for smoking in the form of cigarettes, cigars, pipe tobacco, and flavored shisha tobacco. Some tobacco are consumed in the form of snuff, chewing tobacco, dipping tobacco and snus. Tobacco is instrumental in generating enormous revenue to the national governments and providing employment to billions of people.

10.1.2 Reduction in Yield and Quality Due to Abiotic Stresses

Land plants are surviving in inherently harsh environment ever since their emergence. A large variety of physical or chemical factors are found to be hostile to them,

including low or high temperature, deficient or excessive water, high salinity, heavy metals, and ultraviolet (UV) radiation, among others (He et al. 2018). These stresses, collectively termed as abiotic stresses, are posing problem to agriculture and the ecosystem and accounting for significant crop yield loss (Wang et al. 2003; Wania et al. 2016). In the field, plants are usually exposed to an unpredictable combination of various stresses rather than a single one (Wania et al. 2016), which is even worse in the context of environmental pollution, soil salinization and climate change.

Globally, tobacco crop is cultivated largely in semi-arid and rain-fed areas and is often confronted with various forms of abiotic stresses viz., excess or deficient water, high salinity, high (heat) or low (cold) temperature, heavy metals, salinity, ozone, low and high light intensity, chlorides, heavy metals, ultraviolet radiation etc. The abiotic stresses usually play a negative role in the growth rate of tobacco plant due to different molecular, physiological and cellular response of plant.

Drought is considered the most destructive condition influencing the growth of crop plants consequently leading to decreased yield (Lambers et al. 2008). The amount of damage to yield depends on the severity and duration of stress, plant resistance and plant growth stage (Robertson et al. 2004). Drought stress is a major constraint to tobacco production and yield stability in many rainfed regions of tobacco cultivation.

In addition to its vulnerability to moisture scarcity, tobacco is susceptible to injury from flooding or saturation of the soil with water. Waterlogging lowers the growth and productivity of tobacco. Rainfall accompanied by high winds may cause lodging along with the root damage causing soiling of leaves and make it difficult to manage plants. Flooding of the field for less than 24-h may not significantly affect the growth and yield of the plants whereas flooding for more than 48 h significantly reduced yield even up to 80% than that of the unflooded plants (Campbell 1973; Anuradha et al. 2013) and soil saturation for several days may lead to serious permanent injury or death of the effected plants (Nurhidayati et al. 2017). Flooding also changes leaf chemical quality parameters (Campbell 1973; Anuradha et al. 2013) and found to accelerate flowering time (Higase 1959) which in turn may reduce the yield due to reduction in period of vegetative growth.

Tobacco is a well-known thermophilic crop plant growing under tropical and subtropical region which is highly sensitive to the change of temperature and doesn't require either very high temperature or very low temperature during its growing period (Yamori et al. 2010; Popov et al. 2013; Zhang et al. 2013). Tobacco requires 3–4 months of frost-free period from transplanting to harvesting of the crop (www.fao.org). A slight change in the temperature will make the leaves brittle and affect making it less acceptable. High temperature in tobacco usually disrupts the production of nicotine and other associated pyridine alkaloids (Oeung et al. 2017). The growth of tobacco plants is restricted at the temperature lower than 10–13 °C, affects the morphogenesis of tobacco plants, delays flower initiation and senescence and plants even die when the temperature drops to 2–3 °C (Zhang et al. 2013; Yang et al. 2018). Exposing to 18.5 °C promoted the elongation of petiole and stem, reduced leaf area and increased the angle between leaf and stem of tobacco plants (Yang et al.

2018). Cold stress at harvest period affects agronomic characteristics, leaf quality and curing characteristics (Li et al. 2021).

Higher temperature inhibits the growth and development of tobacco, accelerates flowering and leaf senescence triggering death of plants (Belknap and Garbarino 1996; Yoshida 2003; Lim et al. 2007; Djanaguiraman and Prasad 2010; Gill and Tuteja 2010; Suzuki et al. 2012; Yang et al. 2018) affecting yield and quality. The senescence caused by raise in temperature reduces both the yield and quality of the crop (Chéour et al. 1992; Navabpour et al. 2003; Kim et al. 2011) as early senescing leaves may not grow to their full potential and accumulate the necessary phyto-harmones required for quality (Liu et al. 2015; Nisar et al. 2015).

Light serves as primary energy source in the phototrophic lifestyle of plants. Moderate heat stress and fluctuating light are typical conditions in summer in tropical and sub-tropical regions. The low intensity light deprives the photosynthetic activity and high intensity light damages the photosynthetic apparatus (Tan et al. 2020) leading to lower yields. Exposure to UV-B may lead to either damaging DNA, with subsequent heritable mutations, or by eliciting various regulatory effects that are injurious to plant physiological functions (Lidon 2012).

Weather fleck in tobacco is a leaf spot syndrome induced by air-polluting ozone (Heggestad and Middleton 1959). Weather fleck has caused extensive losses to tobacco growers in the US and Canada since 1955 (Heggestad 1966). The production areas most seriously affected were cigar wrapper in Connecticut and Florida and flue-cured in southern Ontario, Canada. Weather fleck also occurs sporadically in burley, flue-cured, Wisconsin, and Maryland tobacco-producing areas. A number of air pollutants can cause injury to crop plants; evidence, however, indicates that ozone causes the flecking observed on tobacco leaves. Ozone appears to be the primary injury causing pollutant in the Maryland tobacco-producing area as a result of the region's proximity to Washington DC and the high concentration of automobiles, which are considered to be the major source of ozone pollution.

Chlorine in small amounts promotes growth and leaf expansion (Franco-Navarro et al. 2016, 2019) and so improves yield and certain quality factors such as color, moisture content, elasticity, burning and keeping quality of tobacco leaves (McEvoy 1957). However, larger amount of chloride has many adverse effects on the quality of tobacco, so much so that the chloride content in tobacco leaves is considered as a major factor determining the quality of tobacco (Akehurst 1981; Juan and del 1986; Guardiola et al. 1987; King 1990; Chari 1995). An excess level of chlorine produces leaves with poor burning capacity, muddy appearance and undesirable odor as well as highly hygroscopic nature causing discoloration during storage (Karaivazoglou et al. 2005).

Salinity is termed as the total amount of mineral salts dissolved in water and soil (Grattan 2012). Salt stress is the most stubborn one magnified by ever-increasing salinization of arable land worldwide (Munns and Tester 2008; Yuan et al. 2015). More than 20% of cultivated land worldwide is affected by salt stress and is increasing over the time. Salt stress will result in water stress, affecting the plant growth and development, leading to reduced leaf yield (Flowers 2004). Prolonged stress condition leads to death of leaves (Cramer and Nowak 1992). Higher salinity impinges on

plant lifecycle affecting seed germination, seedling establishment, vegetative growth, and flower fertility (Flowers and Colmer 2008; Guo et al. 2012, 2015, 2018).

Soil and water contaminated with heavy metals have now become one of the major constrains to the crop productivity and quality. From the past few decades we are witnessing rapid growth in industrialization and modern agricultural practices which have led to the environmental contamination (Miransari 2011). The increasing population and the continuing food demand add much more to the contamination. Lands are mostly being contaminated due to the use of fertilizers, pesticides, municipal and compost wastes, and also due to release of heavy metal from metalliferous mines and smelting industries (Yang et al. 2005b). Tobacco leaves found to naturally accumulates relatively high levels of heavy metals and particularly cadmium in leaves (Lugon-Moulin et al. 2008; Kaličanin and Velimirović 2012; Ajab et al. 2014; Regassa and Chandravanshi 2016). Cadmium is toxic and non-essential to both plant and humans. The accumulated heavy metals get transferred to human being through cigarette smoking (Jarup et al. 1998; Nordberg et al. 2007; Verma et al. 2010) causing a significantly serious damage on human health (Stojanovic et al. 2004; Norom et al. 2005; Sharma and Dubey 2005; Lugon-Moulin et al. 2006). Heavy metal toxicity symptoms are usually associated with stunted stem and root growth, leaf chlorosis of younger leaves (extends to the older leaves after longer exposure), disturbs phytoharmone levels in the leaves etc. (Fontes and Cox 1998; Reddy et al. 2005; Gangwar and Singh 2011; Srivastava et al. 2012). Excess Zn can also give rise to copper (Cu) and manganese (Mn) deficiencies in plant shoots. Mercury toxicity induces physiological disorder in tobacco plants (Zhou et al. 2007). Excess amount of Chromium (Cr) in the soil results in reticence of plant growth, nutrient imbalance, chlorosis in young leaves, root injury, wilting of tops (Scoccianti et al. 2006) along with inhibition in chlorophyll biosynthesis (Vajpayee et al. 2000). Arsenate (As) acts as an analogue to phosphate and competes in the root zone of plants (Meharg and Macnair 1992).

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

Climate change is resulting in altering weather patterns, rising sea levels, and weather events are becoming more extremes. It is affecting lives in country on every continent and disrupting national economies. The year 2019 found to be the second warmest year on record and the period 2010–2019 was the warmest decade ever recorded. carbon dioxide (CO_2) levels and other greenhouse gases in the atmosphere rose to new records in 2019 (United Nations 2019) Worldwide there is a steady increase in temperature from 1.5 to 2 °C for the past 60 years (IPCC 2019). Warming has resulted in an increased frequency, intensity and duration of heat-related events, including heatwaves in most land regions. Frequency and intensity of droughts has increased in some regions (including the Mediterranean, west Asia, many parts of

South America, much of Africa, and north-eastern Asia and there has been an increase in the intensity of heavy precipitation events at a global scale.

Global warming has led to shifts of climate zones in many world regions, including expansion of arid climate zones and contraction of polar climate zones. Raising temperatures are changing rainfall intensity, flooding, drought frequency and severity, heat stress, dry spells, wind, sea-level rise and wave action, and permafrost thaw with outcomes being modulated by land management. Climate change has been affecting the food security due to warming, changing precipitation patterns, and greater frequency of some extreme events.

Climate change resulting in unpredictable rainfall and increasing temperature with heat waves is going to have remarkable effect on tobacco productivity and quality in view of its sensitivity to these events. Drastic shifting of rainfall pattern and frequent dry spells cause moisture stress especially in critical periods of crop growth, significantly affecting growth, yield and quality of tobacco. Leaf is the important economic product in the tobacco plant. Leaves are harvested when they mature and before they reach senescence. The raise in temperature usually results in earlier senescence of leaves there-by reducing the quality harvest due to insufficient accumulation of quality related phytochemicals. Climatic variability was found to decrease tobacco productivity in Indonesia in 2013 and 2016 (Muttaqin et al. 2019). Both the premature and late-matured tobacco leaves will seriously affect the yield and quality of tobacco crop (Chéour et al. 1992; Navabpour et al. 2003; Kim et al. 2011).

Rapid growth in industrialization and modern agricultural practices in the past few decades has led to the environmental contamination (Miransari 2011). The increasing population and the continuing food demand add much more to the contamination. Most of the lands have been contaminated due to the use of pesticides, fertilizers, municipal and compost wastes, and heavy metals released from smelting industries and metalliferous mines (Yang et al. 2005b). Increased in salinization and heavy metals concentrations affects yield and quality of tobacco. Accumulated heavy metals makes tobacco consumption further harmful.

10.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

Conventional breeding continues to play an important role in improving tobacco productivity under different climatic situations including abiotic stress conditions. Abiotic stresses are complex characters and the success of breeding program primarily depends on the existence of variability for characters that contribute to stress mechanism (Fita et al. 2015). The achievements that can be realized through conventional breeding are limited by non-availability of sources for resistance to abiotic stresses and yield contributing traits, narrow genetic variability, natural barriers of crossing among existing species, longer period for developing stable homogenious lines, undesirable associations between resistant genes and desirable traits either due

to pleiotropic effects of the resistance genes or due to linkage drag effects caused by the presence of deleterious genes linked to gene of interest (Legg et al. 1981; Friebe et al. 1996; Brown 2002; Chaplin et al. 1966; Chaplin and Mann 1978). Recombination suppression within introgressed chromatin (Paterson et al. 1990; Liharska et al. 1996) may interfere in alleviating linkage drag effects through back crossing (Stam and Zeven 1981; Young and Tanksely 1989) and also complicate the efforts to distinguish between pleiotropic and linkage drag effects (Purrington 2000; Brown 2002).

Often the abiotic stresses are controlled by polygenes or many genes with smaller effects and modifier genes with pleotropic effects. Many drought-inducible genes are also induced by salt stress and cold, which suggests the existence of similar mechanisms of stress responses. Hundreds of genes are thought to be involved in abiotic stress responses (Seki et al. 2003; Baloglu et al. 2012). Undesirable linkage of such genes with other deleterious genes makes pooling the resistance genes into a cultivar difficult through classical breeding. The appearance of various abiotic stresses in the crop growth period may vary from year to year and place to place. As the response and vulnerability of various crop stages (seedling, growth, maturity, flowering etc.) to stresses vary it may be difficult to breed lines uniformly resistant for different stages.

Other limitations in conventional breeding are the relatively longer time periods required to combine different target genes and laborious methods of screening/phenotyping segregating generations for abiotic stresses. The success of the abiotic stress resistance breeding depends upon the efficiency of screening techniques for abiotic stresses. The importance of developing reliable screening techniques has been realized very early (Levitt 1972). Plants exhibiting resistance to abiotic stresses can be identified based on their performance under different abiotic stresses after completion of their life cycle under field condition making it difficult to select plants in early stage of their life. Appearance of other stresses during the growth of the plants interfere in clearing assessing the effect of target stress. Such screening related issues are important limitations in achieving progress in resistance breeding.

Various limitations of traditional breeding mentioned can be overcome through the genome designing strategies (Kole 2017). The advancements in the field of genomic designing strategies including molecular breeding, transgenics, genomic-assisted breeding, and the recently emerging genome editing tools are providing a greater promise for improving tobacco for abiotic stress resistance. Whole-genome sequencing and genotyping-by-sequencing methods adopted in tobacco for mapping and trait discovery in recent years may pave the way for obtaining precise information about the genes conferring abiotic stress resistance. The polygenes identified for abiotic stress resistance can be effectively transferred through various molecular breeding methods. Handling of target genes in these methods overcome the issues of undesirable linkages and avoids the transfer of non-target genes, thereby reduce the time taken for elimination of non-target traits. Screening of target traits with tightly linked markers overcome the phenotyping requirements under stress environments and allows early generation screening. The gene editing tools and transgenic

approaches can be of great help in cases where genetic sources of resistance are not available. The emerging genomics-aided techniques including genomic selection, allele mining, gene discovery, and gene pyramiding for developing adaptive varieties holds a great promise for improving tobacco cultivars in terms of abiotic stress resistance in near future.

10.2 Description on Different Abiotic Stresses

10.2.1 Root Characters

Understanding various root characteristics of tobacco viz., manner of branching, the depth of penetration and the lateral spread of the root systems, and of absorbing areas of root under different soil conditions makes it possible to clearly interpret the responses of the plant to the various factors of its environment.

The tobacco has a taproot system that consists of primary, lateral and adventitious roots (Xi et al. 2011). Transplanted tobacco plant possesses an extensive but comparatively shallow fibrous root system (https://ctri.icar.gov.in/for_morphology.php). Most of these roots develop adventitiously from the portion of the main stem buried during transplanting. In general, most of the root system (72% of which were adventitious) of a mature plant filled all the cultivated layer of the A horizon (Gier 1940). The average total length of a mature root system was 260 m with a maximum length of 432 m. The shoot–root ratio ranged from 4.95:1 to 13.0:1 with an average of 10:1. Genotypes differ in length of roots and branching pattern (Jones and Shew 1995).

Bruner (1931) made detail study on the root development in tobacco. He reported that the first structure of the seedling is its main or taproot. The root system of the one month old tobacco plant is succulent and covered with root hairs. Absorption begins at first through the epidermis and soon increases rapidly with the appearance of root hairs even when the root is only a fraction of an inch long. Later, branches appear while the root is still only a few inches long and are soon covered with root hairs, and absorption is greatly increased. The main or taproot and its branches develop in the form of a more or less symmetrical cone which increases in size as the roots develop. As the roots continue to grow the majority of the older absorbing rootlets die and absorption is carried on by the younger rootlets. Those which do not die increase in length and usually in diameter and become permanent roots. The immediate environment determines just which of the rootlets will become permanent roots. The older portions of the roots do not absorb directly and frequently bear no absorbing rootlets. They may be reinvested with absorbing rootlets if the soil moisture is replenished. The absorbing portions of the root system is larger during moist periods owing to an increase in the number and length of absorbing rootlets. The absorbing portion of the root system decreases during periods of drought owing to the fact that the temporary or deciduous absorbing rootlets die much more rapidly

than they are produced. Competition with roots of plants in adjacent areas check the lateral spread of the horizontal roots. This is due, in part at least, to the drying of the soil. This causes most of the main roots to develop in a plane perpendicular to the row. There is a greater tendency for the roots to intermingle if the soil is kept moist as in periods of frequent precipitation.

However, root systems developing from the transplanted tobacco had no taproot and lacks the symmetry (Bruner 1931). Root branches developed from transplanted portion of the original root system and grew at all angles from the base of the plant. Some follows a horizontal course in the moist surface loam (soil) but some grew directly downward or at more or less of an angle. Competition among the horizontal roots caused them to develop less strongly at the extremities as the season progressed. The competition stimulated the development of their longer branches some of which penetrated downward and not infrequently the branch became the main root later in the season. Thus, many roots which appeared to turn rather sharply downward about 2 feet from the plant were not the result of curvatures of the original root but were developed from a portion of a horizontal root and one of its lateral branches. This occurred. Where the distal portion of the horizontal root ceased to function or became unimportant as an absorbing structure.

10.2.2 Drought Tolerance

Drought may be defined as the inadequacy of water availability, including precipitation and soil moisture storage capacity, in quantity and distribution during life cycle of crop plant restricting the expression of genetic potential of the plant. Drought can be the result of an overall decline in rainfall in wet or dry season, a shift in the timing of the wet season, or a strong local warming that exhausts water bodies and soils through evaporation. Drought is considered the most destructive condition influencing the growth of crop plants consequently leading to decreased yield (Lambers et al. 2008). The amount of damage to yield depends on the severity and duration of stress, plant resistance and plant growth stage (Robertson et al. 2004). Drought stress is a major constraint to tobacco production and yield stability in many rainfed regions of tobacco cultivation. Drastic shifting of rainfall pattern and frequent dry spells cause moisture stress especially in critical periods of crop growth, significantly affects growth, yield and quality of tobacco. Drought stress limits the growth and economic yield of tobacco through reduction in leaf growth, chlorophyll concentration, soluble protein concentration, stomatal conductance, accelerating senescence of leaves and reducing the rate of photosynthesis etc.

10.2.3 Flooding and Submergence Tolerance

Tobacco plants are mostly cultivated in a dry climate. Tobacco plants require dry land conditions for 2–3 months after planting to harvest leaves and for the ripening process (Muttaqin et al. 2019). One risk of tobacco cultivation in general is the high rainfall causing waterlogging. This type of environmental stress might happen because of unpredictable season occurring in tropical region and global climate change as a consequence of rapid growing industries all over the world.

Tobacco is the most susceptible crop to injury from flooding or saturation of the soil with water. Waterlogging lowers the growth and productivity of tobacco, which is very sensitive to an excess of water. It exhibits two types of reaction to flooding, immediate but temporary wilting accompanying temporary flooding, and severe permanent injury caused by longer periods of flooding (Kramer 1951; Kramer and Jackson 1954; Campbell 1973). If the soil is suddenly saturated by a downpour of rain and the sun later shines bright and hot, sudden wilting of the leaves, often termed "flopping" by tobacco growers, sometimes occurs. This sudden wilting occurs where drainage is slow and the soil remains saturated for at least a few hours after a rain. High air temperatures and bright sun accentuate this sudden wilting and its occurrence may depend also on the condition of the plants, apparently being much more severe if the tobacco has been growing rapidly and therefore is somewhat soft and succulent. If high winds accompany the rainfall, blow over may occur along with the root damage. Lodged or blown-down tobacco can be difficult to manage. If the excess soil moisture drains away within a few hours the plants usually recover from this type of wilting with little or no permanent injury. The growth and yield of the plants may not be significantly affected if plants are flooded for less than 24-h, whereas flooding for longer than 48 h significantly reduced yield even up to 80% than that of the unflooded plants (Campbell 1973; Anuradha et al. 2013).

10.2.4 Light Stress

Light serves as primary energy source in the phototrophic lifestyle of plants. The photoreceptors of plants perceive the light and regulate different metabolic processes through gene expression (Gyula et al. 2003; Kami et al. 2010; Jenkins 2014). The changes in light conditions directly affect the photosynthetic reactions within chloroplasts. The detrimental effect of high or low intensity light on biological and metabolic processes of plant is denoted as light stress. The low intensity light deprive the photosynthetic activity and high intensity light damages the photosynthetic apparatus. The decline of photosynthetic activity due to intense incoming light is known as photoinhibition. The shuttle changes in light quantity and quality primarily cause imbalances in the light reactions of photosynthesis and the carbon fixation reactions. However, light stress is not a major issue of concern in tobacco cultivation and currently researchers are not seriously attempting to breed tobacco for light stress.

10.2.5 UV Stress

Plants are exposed to ultraviolet-B (UV-B, 280–320 nm) at varied intensities based on the solar angle and spread of stratospheric ozone layer in the specified region. Although UV-B is only a minor component of the total solar radiation (0.5%), increase in its intensity has devastating effects on biological systems. In the experiments conducted with tobacco seedlings (*N. tabacum* L. cv. K326), exposure to UV-B stress increased the carotenoid synthesis capability of plants (Shen et al. 2017). The plants could deplete the carotenoids to scavenge excess reactive oxygen species (ROS) at high UV-B radiation levels, which protects the tobacco plant from oxidative damage caused by UV-B stress. While increasing the photosynthetic efficiency it was found that expression of the carrot lycopene β-cyclase (DcLCYB1) in *N. tabacum* cv. Xanthi resulted in increased carotenoid accumulation, faster plant growth, early flowering and increased biomass there-by higher yields in constant and fluctuating light conditions (Juan et al. 2020). Further, UV stress also induces physiochemical changes in tobacco leaf, reduces the amount of wax deposited on the adaxial leaf surface and also alters the density of trichomes in tobacco leaf (Barnes et al. 1996). Breeding tobacco for UV stress is not a priority to tobacco researchers as UV stress is not a major limiting factor in tobacco cultivation.

10.2.6 Weather Fleck (Ozone Pollution)

Weather fleck in tobacco is a leaf spot syndrome induced by air-polluting ozone (Heggestad and Middleton 1959). Weather fleck has resulted in extensive losses to tobacco growers in the US and Canada since 1955 (Heggestad 1966). The two production areas most seriously affected have been cigar wrapper in Connecticut and Florida and flue-cured in southern Ontario, Canada. Weather fleck also occurs sporadically in burley, flue-cured, Wisconsin, and Maryland tobacco-producing areas. A number of air pollutants can cause injury to crop plants; evidence, however, indicates that ozone causes the flecking observed on tobacco leaves. Ozone appears to be the primary injury causing pollutant in the Maryland tobacco-producing area as a result of the region's proximity to Washington, DC and the high concentration of automobiles, which are considered to be the major source of ozone pollution.

10.2.7 Chloride Stress

Among mineral nutrients, chlorine is recognized as an essential micronutrient in tobacco cultivation. Tobacco is known to accumulate chloride very rapidly in considerable amounts, and an amount up to 100 g Cl kg^{-1} leaf dry matter have been observed. Chorine in small amounts results in promoting growth, leaf expansion, a

better hydration state, reduced transpiration, higher water use efficiency (WUE), and water saving (Franco-Navarro et al. 2016, 2019) and so improve yield and certain quality factors such as color, moisture content, elasticity, burning and keeping quality of tobacco leaves (McEvoy 1957). However, larger amount of chloride has many adverse effects on the quality of tobacco, so much so that the chloride content in tobacco leaves is considered as a major factor determining the quality of tobacco. An excess level of chlorine produces leaves with poor burning capacity, muddy appearance and undesirable odor as well as highly hygroscopic nature causing discoloration during storage (Karaivazoglou et al. 2005). The threshold value for chloride in a good and acceptable tobacco leaf is usually set at below 1.5% (Chari 1995), the values greater than 2% inhibit the burning properties of tobacco (Akehurst 1981; Juan and del 1986; Guardiola et al. 1987; King 1990). Tso (1990) reported that various soil and fertilization conditions, as well as tobacco type, variety and methods of harvesting may contribute to the differences in the absorption, distribution of chloride with respect to stalk positions and the total leaf chloride content.

10.2.8 Salinity Stress

Salinity is termed as the total amount of mineral salts dissolved in water and soil (Grattan 2002). More than 20% of cultivated land worldwide is affected by salt stress and is increasing over the time. It is mainly related to increase in Na^+ and Cl^- ions and decrease in K^+ and Ca^+ ions in plants (Perez-Alfocea et al. 1996; Shilpim and Narendra 2005). Salt stress will lead to water stress there-by affecting the leaf growth and development. Salinity stress negatively influences the cell division and expansion as well as stomatal opening and closing (Flowers 2004). In tobacco, soil salinity is known to reduce the plant growth through osmotic stress followed by ion toxicity. The salt stress in tobacco can be divided into ion toxicity (such as destroying plasma membrane structure, hindering the absorption of mineral elements, etc.) and the secondary stress effect (oxidative stress, drought stress, etc.) (Sharma et al. 2019).

10.2.9 Heavy Metal Stress

The tobacco farmers are bound to use huge amount of fertilizers and pesticides which contain high levels of metals (Karaivazoglou et al. 2007; Lecours et al. 2012). The main reason of heavy metals is the phosphate fertilizers utilized in the tobacco cultivation. The levels of metal accumulation in the leaves are found to vary (Lugon-Moulin et al. 2006) based on the area in which tobacco is cultivating. Vardi and Venkatrayulu (2019) reported the contamination of water samples with heavy metals namely, Arsenic, Lead, Cadmium, Mercury, Iron, Manganese, Copper, and Zinc which was above the WHO standards. Heavy metal toxicity symptoms are usually associated with stunted stem and root growth, chlorosis in younger leaves which

extends to the older leaves after long term exposure (Gangwar and Singh 2011; Srivastava et al. 2012).

10.2.10 Traditional Breeding Methods Addressing Abiotic Stresses

Tobacco is a self-pollinated crop with 5–10% out-crossing. Hence, all the breeding methods such as introduction, mass selection, pure line breeding, pedigree method, back cross breeding, mutation breeding, interspecific hybridization etc. that are commonly used in self-pollinated crops are being used in tobacco breeding (Bowman and Sisson 2000; Sarala et al. 2012). However, development and release of resistant cultivars for specific abiotic stresses is not there in tobacco. In view of selection of plants in low moisture regimes and having chloride contents below 1 ppm in breeding programs may be ensuring in developing cultivars that can adopt to water and chloride stress to certain extent (Sarala et al. 2012).

In order to get higher and stable yields under different abiotic stresses, the inherent capacity of tobacco genotypes need to be improved. However, development of stress tolerant tobacco cultivars requires thorough understanding of plant responses to stress environment. Information on availability of stress resistance sources, understanding the genetics and inheritance pattern of genes involved in stress resistance, molecular mechanisms conferring resistance and genome sequences associated with abiotic stress resistance are essential inputs in the development of abiotic stress resistant tobacco cultivar.

The first major category of breeding for abiotic stress environments is the indirect method, this approach attempts to breed for high yield and quality under several environments ranging from optimum conditions to stress conditions. The genotypes selected with high yield and quality under optimum conditions will also excel under drought conditions. As such, in this approach selections are not based directly on stress factors. There is evidence of existence of high positive correlation between performance of genotypes in optimum and stress conditions (Johnson and Frey 1967). The selections having high yield and quality over environments are further evaluated under a range of environments and those selections showing high performance with stability can be released for its cultivation in stress prone areas.

In order to overcome the problems of indirect breeding, second category for breeding approach for abiotic stress resistance (drought) has been advocated (Hurd 1971) in which test materials are evaluated in deliberately chosen testing sites that represent drought conditions reliably and uniformly. It is obvious that uniformity of drought conditions in field trials cannot be imposed while making selection among the genetic materials as drought is highly unpredictable and varies over the years and locations, thus resulting in reduced effectiveness of selection especially for yield which has low heritability.

The third category of abiotic stress resistance breeding is through incorporation of characters that contribute to drought mechanism into a high yielding variety. The choice of the character/characters to be incorporated will depend mainly on the importance of the character in enhancing abiotic stress resistance without much compromise on yield; the ease, speed, inexpensive and accuracy in measurement of the characters; targeted trait should have high heritability than yield and positively correlated with yield and the character should be reasonably stable over the time and should withstand minor environmental fluctuations.

Methodology of the incorporation of such characters depends upon the gene controlling the character and heritability. Characters controlled by oligogenes that are simply inherited, and if the aim is to transfer few characters backcross breeding is the usual choice. Characters controlled by polygenes that are considerably affected by environmental factors pedigree method are followed. A combination of drought contributing characters rather than a single character is much more useful selection criteria for drought resistance (Lundlow and Muchow 1990). When such is the aim for simultaneous selection of drought contributing characters having different heritability's, as is usually the case in many breeding programs, modification of bulk method called single seed descent is advocated. Jinks et al. (1977) while evaluating the random sample of 59 F_1 of *N. rustica* lines through single seed descent method had isolated superior recombinant lines for flowering date and plant height and demonstrated that the mean performance and environmental sensitivity were largely under control.

The success of any of the above breeding approaches and methods depends upon the efficiency of screening techniques of drought resistance. The importance of developing reliable screening techniques has been realized very early (Levitt 1972). Numerous screening techniques for drought under laboratory and field conditions have been developed and breeder can adopt any of the techniques depending upon the situation. Since increasing yield is the ultimate goal of any plant breeding program, breeders emphasize on higher yields under moisture stress conditions. Therefore selections based on drought index, which provides a measure of drought based on loss of yield under drought condition verses optimum/stress condition is advocated for drought screening (Clark et al. 1984; Ndunguru et al. 1995). At ICAR-Central Tobacco Research Institute (CTRI), breeding program for drought has been initiated utilizing drought resistant entry, MRS 3 as one of the parent. The material is being screened under optimum conditions coupled with in vitro screening for germination under high molecular weight poly-ethylene glycol (PEG).

10.2.11 Use of Morphological Markers

Morphological markers in tobacco are related to easily identifiable variation in different plant, leaf, flower, capsule and seed characters (Sarala et al. 2018a, b). These markers can be easily scored and doesn't require sophisticated equipment or

preparatory procedures. Breeding for abiotic stresses aims to incorporate morphological characters that directly or indirectly contribute to resistance/tolerance mechanism into a high yielding variety. The choice of the character/characters to be incorporated will depend mainly on the importance of character in enhancing abiotic stress resistance without much compromise on yield; the ease, speed, inexpensive and accuracy in measurement of the characters; targeted trait should have high heritability than yield and positively correlated with yield and the character should be reasonably stable over the time and should withstand minor environmental fluctuations.

Morphological, anatomical and compositional characters identified to be associated with abiotic stress tolerance/resistance (especially drought) in tobacco that can be used as morphological markers to develop a model plant conferring abiotic stress resistance are discussed below.

Increased rooting depth and rooting density have been found to correlate positively with resistance to water deficit (Tuberosa et al. 2002). The length, weight, volume, penetrative ability, density of plant roots were reported to be associated with drought resistance (Tuberosa 2012). When water reserve exists at depth, a decrease in the cytokinin (CK) level or a reduction in CK signaling can lead to an enlarged root system (Macková et al. 2013) that reaches the water resources. When moisture reserves are confined to the upper layer of soil, number of lateral roots gain importance than deep root system. Breeding for small xylem vessels in the seminal roots has been suggested as a means for increasing the resistance (Passioura 1983). Increased root/shoot ratio is also associated with drought resistance (Bliss et al. 1957).

As tobacco varieties are bred for higher leaf yields, mechanisms that regulate transpiration improves WUE and confers drought resistance in plants. Morphological responses like, narrow and thick leaves (Nobel 1980; Abrams et al. 1990), leaf area (Turner 1986; Pereira and Chaves 1993), surface leaf rolling, increasing cuticular waxes deposition (Cameron et al. 2006), hairs on leaf surface, covering with trichomes on leaves, mid-veins, stalks, and floral parts of many *Nicotiana* species (Goodspeed 1954) aid in reduction of transpiration. Low stomatal frequency can reduce potential transpiration and improve WUE (Wilson 1975; Wang et al. 2012; Dias de Oliveira et al. 2013). Stomata size, distribution and sunkeness plays a critical role in regulation of transpiration.

Trichomes present in tobacco play several roles in the defense against abiotic stresses such as moderation of leaf temperature or water loss through increased light reflectance, sequestration and compartmentalization of heavy metals etc. (Wagner 1991; Harada et al. 2010). Trichomes also play an important role in ion and metal homeostasis of plants. Hence, recording observations on trichome densities is important in identifying resistant genotypes in breeding programs aiming at multi stress-tolerant genotypes.

Altered anatomical properties like thicker palisade tissue, a higher ratio of palaside to spongy parenchyma thickness and a more developed vascular bundle sheet reduce excess water loss and enhance water holding ability there-by improves drought tolerance (Esau 1960; Guha et al. 2010). The fortified sclerenchyma can reduce the damage from wilting and protect plants from direct light radiation (Terashima 1992).

The phenomenon of the accumulation of various organic and inorganic substances such as sugars, polyols, amino acids, alkaloids and inorganic ions in the cytochylema reduces the osmotic potential, increases cell water retention and elasticity of cell (Morgan 1984; Rhodes and Samaras 1994). Such osmotic adjustment, sustains cell structure and photosynthesis, delays leaf senescence and improves root growth under stress conditions.

Tobacco has evolved a C3 path way for carbon dioxide and carbohydrate fixation which is not as efficient as other two path ways viz., crassulacean acid metabolism (CAM) and C4, because of photorespiration. Photorespiration increases with the temperature and under moisture conditions (Rivero et al. 2009; Huang et al. 2016). A balance between growth and carbon supply is achieved through a complex regulatory network in which sugars (e.g., glucose, sucrose and starch) and phytohormones, mainly abscisic acid (ABA) and cytokinins (CK) perform central roles (Shinozaki and Yamaguchi-Shinozaki 1996; Rolland et al. 2006; Havlov et al. 2008; Nishiyama et al. 2011). Under moisture stress conditions roots of plants triggers a huge increase in *de nova* synthesis of ABA (Sauter et al. 2001) and transported mainly to leaves as an intercellular messenger and recognized by guard cells which trigger stomatal closer via intercellular single transduction and weakening the metabolic activities related to plant growth (Boursiac et al. 2013). The influence of ABA has multiple effects on drought response encompassing the regulation of stomatal closure, channel activities in guard cells, transcriptional levels of calmodulin protein and the expression of some ABA responsive genes (Cocucci and Negrini 1988; Rabbani et al. 2003). Researchers are paying attention to improve photosynthesis efficiency for increasing yields under different conditions including stresses (Zhu et al. 2010; Long et al. 2015; Ort et al. 2015).

Majority of the stresses affect plant growth, development and morphogenesis. Recording observations on relative plant growth rates and morphogenesis patterns are essential in identifying the effect of various stresses and resistant genotypes. Time taken for stress symptom occurrence after the stress incidence, its relative severities, symptom progression and recovery patterns after stress amelioration are important morphological parameters in identifying abiotic stress resistant genotypes.

10.2.12 Limitations and Prospect of Genomic Designing

The genome designing strategies overcome the limitations of conventional breeding as they deal at the level of genomes and manipulate the gene sequence to achieve desired phenotypic traits. Transfer of desired traits from tertiary gene pools and other unrelated sources to cultivated tobacco can also be successfully achieved through trans- and cis-genesis approaches involving gene mapping, identification, gene transfer, gene editing etc. With the rapidly evolving technological advancements, marker and genome assisted breeding approach is going to accelerate the progress made in breeding programs. Targeted modification or designing of plant

genome including addition of alien genes will accelerate the tobacco varietal developmental process through precise manipulation of gene functions for higher yields and stress resistance.

Published draft genome of *N. tabaccum* and a few wild species and data sharing and analysis platforms (databases) available in recent times, made it possible to use innovative bioinformatics tools for the in depth study of genomes and their comparative genomic analysis. Such studies are helping to understand genes, their sequences and linked molecular markers for target traits. This information can successfully be utilized to edit the genome sequences with rapidly evolving precise gene editing tools viz. meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), homing endonucleases, CRISPR/Cas 9 etc. Gene editing technologies at present do suffer from lower specificity due to their off-targets side effects (Khan 2019). High density of molecular maps and genome information are now offering scope for providing the knowledge of linked molecular markers and quantitative trait loci (QTLs) that are either tightly linked or present within the target gene (s) and also allow map-based cloning of desirable traits. Linked markers and QTLs identified in tobacco for various abiotic stress responsive genes are going to pave the way for marker assisted breeding for resistance to abiotic stresses. The available information on linked markers and traits can be effectively used in estimating the breeding value of individuals in genomics aided breeding and accordingly desired plants can be selected.

Though genetic engineering (GE) tools offer a number of advantages, they do have certain limitations. Time-consuming and complicated protocols, potential tissue damage, incorporation of DNA of selection marker in the host genome, and low transformation efficiency etc. are some of the limitations of GE technologies. Compared to tradition breeding, genome designing techniques are resource intensive and require technology expertise for handling the protocols and processes.

10.3 Genetic Resources of Resistance Genes

Availability of genetic resources with stable and heritable resistance factors for abiotic stresses can facilitate breeding resistant varieties. The gene pools consist of easily crossable tobacco lines and *Nicotiana* species are to be explored for such variability. In case of non-availability of sources of resistance in any of these gene pools, variability need to be created through mutations or incorporated through genome designing approaches.

Currently, large number of cultivated tobacco varieties and around 83 *Nicotiana* species are available (Lewis 2011; Berbeć and Doroszewska 2020). Taxonomy Browser of National Centre for Biotechnology Information (NCBI) lists around

92 *Nicotiana* species and varieties (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi). 307 records of tobacco varieties and *Nicotiana* species and available in The Plant List database (http://www.theplantlist.org/tpl1.1/search?q=Nicotiana). The International Plant Name Index providing 440 records with the keyword 'Nicotiana' through its database (http://www.ipni.org/ipni/plantnamesearchpage.do). Large number of these species are reliable sources of resistance to various stresses (Lewis 2011). Wild *Nicotiana* species are good sources of cytoplasmic male sterility (CMS) for developing male-sterile isolines of inbred lines and cultivars. CMS is a prerequisite in tobacco for technically feasible and economically viable seed production of hybrid varieties. Various available sources of resistance in tobacco genetic resources are discussed below.

10.3.1 Primary Gene Pool

The primary gene pool includes genotypes that are crossable and produce fertile offspring with the cultivated tobacco. They may be cultivated species and in wild gene pools. The cultivated gene pool comprises of commercial varieties, as well as landraces. Wild gene pool comprises closely related species and putative ancestors that have fair degree of crossability with the cultivated tobacco. Large number of varieties are developed by breeders in different countries and fairly large collections of germplam are available in *N. tabacum* and *N. rustica* that can be explored for the identification of abiotic stress resistant factors as gene transfers from such sources is easy.

10.3.2 Secondary Gene Pool

The secondary gene pool refers to crop wild relatives that can cross with the cultivated tobacco at least to some extent to produce some fertile offsprings. These sources are distinct from the cultivated species and include closely related species, primitive cultivars, old land races evolved and adopted to different environments and hence, valuable source for resistance to abiotic stresses. *N. tabacum* is found to hybridize with majority of *Nicotiana* species (58 no.) directly or through sister *Nicotiana* species (Berbeć and Doroszewska 2020). *N. tabacum* is found to yield inviable hybrids with N. *africana*, *N. excelsior*, *N. goodspeedii*, *N. gossei*, *N. maritima*, *N. megalosiphon* and *N. velutina* when crossing at 28 °C (Tezuka et al. 2010). However, Type II hybrid lethality showing the characteristic symptoms of browning of hypocotyls and roots observed in these crosses can be suppressed at higher temperatures (34–36 °C). Utilization of genes from these materials is tedious due to incompatibility and undesirable linkages. Genomic tools can assist in overcoming such difficulties.

10.3.3 Tertiary Gene Pool

More distantly related crop wild relative species are included in this pool. *N. tabacum* had no hybridization records with 14 *Nicotiana* species (*N. azambujae, N. acaulis, N. ameghinoi, N. paa, N. cutleri, N. longibracteata, N.* spegazzini*, N. faucicola, N. fatuhivensis, N. heterantha, N. monoschizocarpa, N. stenocarpa, N. truncata,* and *N. symonii*) (Berbeć and Doroszewska 2020). Hybrid lethality in crosses with incompatible *Nicotiana* species may be due to the genes in both the S and T sub-genomes of *N. tabacum* (Tezuka and Marubashi 2012). Specific techniques such as bridge crossing, ovary/ovule culture, embryo rescue, various sorts of treatments of male and/or female flower parts, partial genome transfer (chromosome addition and/or substitution lines, translocation breeding, mutagenesis, cell fusion, etc.) chromosome and genome manipulation (polyploidization or haploidization), exchange of nuclear and cytoplasmic genomes (mitochondrial and/or chloroplastic), grafting, marker-assisted breeding (MAB), tissue culture and genetic engineering are needed to transfer genes from such pools (Weil et al. 2010).

10.3.4 Artificially Induced/Incorporated Traits/Genes

Creation of mutations (physical and chemical mutagens), genetic engineering for transfer of alien genes, gene manipulation and genome editing technologies are to be adopted in developing resistant cultivars when source of resistance is not available in any of the above pools.

10.4 Glimpses on Classical Genetics and Traditional Breeding

10.4.1 Classical Mapping Efforts

Very few classical studies were reported in tobacco. Clausen and Goodspeed (1926) established that one of the two types of monosmics (haplo-C, then called "corrugated"), involved the chromosome in which the basic color factor, *Wh*, is located. Anderson and Dorothea (1931), East (1932), and Brieger (1935) reported linkage between a pollen color factor and the sterility factors. Later, Brieger (1935) established the first two linkage groups 1. self-sterility allele (*S*) and lethallity (*I*) 2. *C* is the basic gene for anthocyanin color and a recessive gene causing a peculiar type of growth *cr* (crassa) based on the linkage data on in *N. langsdorfii* and *N. sanderae.* Smith (1937) confirmed the existence of linkage between self-sterility and pollen anthocyanin color in tobacco.

Later, Clausen and Cameron (1944) established location of 18 genes on nine chromosomes through the transmission studies between monosomics and mendelian characters using complete set of 24 monosmics. However, due to its allopolyploid nature (Suen et al. 1997; Narayanan et al. 2003) genetic linkage maps are not fully developed in tobacco.

10.4.2 Limitations of Classical Endeavors and Utility of Molecular Mapping

Mapping based on morphological markers is tedious and time taking and genes governing quantitative traits cannot be mapped (Worland et al. 1987). To make gene maps more comprehensive it would be necessary to find characteristics that were more distinctive and less complex than visual ones. But, only a fraction of the total number of genes in tobacco exist in allelic forms that can be distinguished conveniently making it difficult to construct classical maps. One of the reasons why our knowledge of the details of inheritance in tobacco was so meager, is because of the prevailingly quantitative or semi-quantitative nature of majority of characters including flower color in tobacco (Clausen and Cameron 1944). Abiotic stress responses is the result of action of numerous genes with major and minor effects with low heritability and are influenced by environmental factors of the gene which adds to the genotyping woes.

Recent enormous progress in the field of biotechnology, especially with the advent of DNA markers, QTL mapping techniques, genome sequencing techniques, gene/genome editing techniques and genome wide association mapping techniques, identification and mapping of candidate genes/markers conferring abiotic stress resistance/tolerance is becoming more feasible.

However, in comparison to the other Solanaceae crops such as the tomato, potato, and pepper plants, molecular marker development and genetic map construction in tobacco have lagged behind (Tanksley et al. 1992; Barchi et al. 2007). The molecular marker based maps can be effective anchoring points for identification of linked traits for their isolation, cloning and also for use in marker-assisted breeding.

10.4.3 Breeding Objectives

The tobacco breeding mainly aims at enhancing leaf yield potential of the cultivar in addition to maintaining leaf quality, and resistance to biotic and abiotic stresses. Numerous studies have identified plant characters that are associated with various abiotic stress responses. For example, increased root depth and root density has been found to correlate positively with resistance to water deficit (Tuberosa et al. 2002). Increased root/shoot ratio is also associated with drought resistance (Champoux

et al. 1995; Tavakol and Pakniyat 2007; Ali et al. 2009; Pallardy 2010). Morphological responses like, narrow and thick leaves (Nobel 1980; Abrams et al. 1990), leaf area (Turner 1986; Pereira and Chaves 1993), surface leaf rolling, increasing cuticular waxes deposition (Cameron et al. 2006), hairs on leaf surface, covering with trichomes on leaves, mid-veins, stalks, and floral parts of many *Nicotiana* species (Goodspeed 1954) aids in reduction of transpiration. Trichomes are involved in the moderation of leaf temperature or water loss through increased light reflectance (Wagner 1991). Stomata size, distribution and sunkeness plays a critical role in regulation of transpiration. Low stomatal frequency can reduce potential transpiration and improve WUE (Wilson 1975; Wang et al. 2012; Dias de Oliveira et al. 2013). Altered anatomical properties like thicker palisade tissue, a higher ratio of palaside to spongy parenchyma thickness and a more developed vascular bundle sheet reduce excess water loss and enhance water holding ability there-by improves drought tolerance (Esau 1960; Guha et al. 2010). The fortified sclerenchyma can reduce the damage from wilting and protect plants from direct light radiation (Terashima 1992). Menser and Street (1962) showed that N nutrition critically affected the weather fleck susceptibility of Catterton tobacco. Fleck and N supply were related inversely. Selection for these associated traits in the desired direction, positive or negative, would yield desired results.

10.4.4 Classical Breeding Achievements

Traditional tobacco breeding aimed at developing improved tobacco varieties with higher yield, better leaf quality, resistance to biotic and abiotic stresses. Significant progress has been made over the years in enhancing the tobacco leaf yield through both varietal and hybrid development, in addition to improving disease and insect resistance without significantly sacrificing in ease of curing. However, success in case of abiotic stress resistance/tolerance is relatively low.

Janardhan et al. (1994) identified Bell No. 10, Bigorinico, Cocker 128, F. 207 and F. 212 as tolerant to drought using sprinkler line-source technique under field conditions during rain-free post monsoon season. Sarala et al. (1998) identified tobacco genotypes, Cy 113, Cy 118, Kanchan, L 621, VA 21 and CM 12 as drought tolerance lines under cyclic water stress (Sarala et al. 1998). Cultivated Varieties viz., Zhubo-1, G 80, K346 Sahyadri, N-98, Tugabhadra, Anand-119, GT-4 CTRI Special, Jayashree, 16/103, Godavari Special, Hema, VT1158, Rathna etc. are found to be drought resistant/tolerant (ICAR-CTRI 2021). FCV tobacco entries, FCR-23 and FCR-15 recorded higher pollen and seed germination under higher PEG concentration which indicates their drought tolerance capacity (ICAR-CTRI 2016).

Povilaitis and White (1966) used a segregating population of flue cured 'Delcrest' to develop fleck tolerant 'Delcrest 66'. McKee (1968) developed Maryland 64 by crossing Catterton X Wilson and selected for an intermediate, high yielding type. His efforts led to the most fleck-resistant of the Maryland cultivars currently grown although obtaining higher fleck resistance was not his main objective.

Nurhidayati et al. (2017) identified tobacco varieties, Kemloko 3 (index value of 0.03), Paiton 2 (index value of 0.18), and Kemloko 2 (index value of 0.42) as resistant to water logging stress based on the sensitivity index. FCV cultivars viz., FCJ-11 and FCR-15 found to withstand wet foot to certain extent (Sarala et al. 2020).

10.4.5 Limitations of Traditional Breeding and Rationale for Molecular Breeding

Most of the stress tolerance traits are mainly quantitative trait loci and greatly influenced by environment, thus making selections difficult (Anderson et al. 2014). The mechanisms of abiotic stresses like drought tolerance are highly complex and recent advances have provided insight into plant gene regulatory network system, which is mainly composed of inducible-genes (environmental factors and developmental cues), expression programming and regulatory elements (cis-element and trans-element), corresponding biochemical pathways and diverse signal factors (Tang et al. 2003; Wang et al. 2003; Zhu 2003; Munns 2005). Many drought-inducible genes are also induced by salt stress and cold, which suggests the existence of similar mechanisms of stress responses. Hundreds of genes are thought to be involved in abiotic stress responses (Seki et al. 2003; Baloglu et al. 2012). The biggest challenge in traditional breeding is the environmental interaction and low heritability of the genes involved in regulating resistance/tolerance mechanism which drastically hinders the progress of incorporation of characters of abiotic stress resistance and may not reflect the desired yield coupled with resistance due to difficulties in selections owing to considerable variation in the imposition of stresses in field conditions.

The major limitation in traditional breeding for abiotic stress response in tobacco is undesirable gene association is polygenic inheritance coupled with low heritability of the genes involved in abiotic resistance/tolerance reaction.

With the advent of genome designing techniques like, marker-assisted selection (MAS), plant transformation, various gene editing tools etc. and identification of several candidate genes with major effects, is possible to develop tobacco cultivars having resistance/tolerance reaction to various abiotic stresses.

10.5 Brief on Diversity Analysis

Lack of diversity in cultivated crops can lead to crop losses due to reduced flexibility of varieties to adapt to changing environmental conditions such as increasing temperatures or salinity and to combat infestations of new strains of biotic stresses (Moon et al. 2009a). Hence, genetic diversity analysis of germplasm is essential for identifying sources for economically important traits and diverse parentals to

create maximum genetic variability in the breeding populations for effecting selection in breeding (Barrett and Kidwell 1998). Deploying tobacco varieties developed from diverse genetic backgrounds insulate the crops from changing environmental stresses. Thus, genetic diversity analysis is an essential step for continued progress in breeding as well as for adaptation to future environmental challenges.

10.5.1 Phenotype-Based Diversity Analysis

Phenotypic diversity in terms of morphological, karyotypical and physiological characters have been regularly studied in tobacco germplasm (Goodspeed 1954; Zhang 1994; Lu 1997). Diversity is found to exist in tobacco germplasm for several agro-morphological traits (Zhang 1994; Wenping et al. 2009; Zeba and Isbat 2011; Baghyalakshmi et al. 2018; Sarala et al. 2018), chemical and cytological traits (Tso et al. 1983; Okumus and Gulumser 2001; El-Morsy et al. 2009; Darvishzadeh et al. 2011). Agro-morphological traits are found to vary with environment and their diversity estimates are affected under different environments (Lu 1997). Studying the diversity of morphological characters for importing abiotic stress resistance and yield contributing traits is essential for breeding abiotic stress tolerance in tobacco.

The tolerance/resistance mechanism to different abiotic stresses is now being extensively studied through high throughput phenotyping where the system quantifies a number of traits in a population with automated image collection and analysis. This technology can effectively be utilized in breeding genotypes for abiotic stresses to its non-destructive sampling methods, rapid screening of larger population under artificially created abiotic stress conditions (Buschmann and Lichtenthaler 1998; Goggin et al. 2015). Such high throughput phenotyping systems could possibly reduce the amount of labor and screening time for identifying plants that are tolerant and have desirable traits.

10.5.2 Genotype-Based Diversity Analysis

Limited information has been available confirming the relationship between morphological variability and genome diversity in cultured tobacco. In view of this, attempts made to examine the degree of relatedness, among tobacco cultivars and diversity of germplasm, based on variability at DNA level. As about 77% of the total genomic DNA content is composed of repetitive sequences in tobacco, the remaining non-repetitive sequences part is responsible for variability in morphological and quality traits (Narayan 1987).

The advent of different types of molecular markers over the last two decades has revolutionized the entire scenario of biological sciences including tobacco (Liu and Zhang 2008). These markers are abundantly available throughout the genome and offer advantages such as highly polymorphic nature, codominant inheritance,

easy access, easy and fast assay, high reproducibility and easy exchange of data between laboratories. Molecular markers provide a relatively unbiased estimation of genetic diversity in plants. DNA-based molecular markers have acted as versatile tools and have found their own position in various fields like characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, genome comparisons, gene mapping, quantitative trait loci analysis, marker-assisted breeding diagnostics, etc.

Molecular markers such as restricted fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeat (SSR), single-nucleotide polymorphism (SNP), inter simple sequence repeats (ISSR) etc. have been employed in studying genetic diversity, gene mapping and marker-assisted breeding of tobacco. RFLP markers were the first molecular markers used in tobacco research specially to study the function of few cloned genes (Bretting and Widrlechner 1995). Invention of polymerase chain reaction (PCR) technology and PCR-based markers such as RAPD and AFLP emerged in the beginning of nineties and later microsatellite markers were used by different workers to study genetic diversity in tobacco. Compared to RFLP, these PCR based markers are preferred because of the relative ease with which PCR assays can be carried out. Both RAPD (Xu et al. 1998; Del Piano et al. 2000; Evanno et al. 2005; Zhang et al. 2005, 2008; Arslan and Okumus 2006; Sarala and Rao 2008; Sivaraju et al. 2008; Denduangboripant et al. 2010; D'hoop et al. 2010) and AFLP (Huang et al. 2008; Zhang et al. 2008; Chuanyin et al. 2009; Liu et al. 2009) were used to analyze the genetic diversity and varietal identification in tobacco.

Soon after the discovery of simple sequence repeat (SSR) markers in late 90s and the beginning of twenty-first century, they became markers of choice as they could be able to eliminate all drawbacks of earlier DNA marker technologies (Jafar et al. 2012). Considering that the genetic diversity between tobacco cultivars (particularly between those of the same type) is very limited (Del Piano et al. 2000; Rossi et al. 2001; Julio et al. 2006) and that cultivated tobacco is a tetraploid species with a very large genome (Livingstone et al. 1999; Ren and Timko 2001; Doganlar et al. 2002), making the development of PCR-based molecular markers generally inefficient. However, Bindler et al. (2007) for the first time employed around 637 functional SSR markers (out of which 282 were highly polymorphic) for variety identification. Since then, SSR markers are being regularly used in estimating the diversity in tobacco. An additional set of 5,119 new and functional SSR markers markers were developed for mapping and diversity studies by Bindler et al. (2011). Later, Tong et al. (2012) developed another set of SSR markers [including 1365 genomic SSRs and 3521 expressed sequence tag (EST)-SSRs] that slightly overlapped the set published by Bindler et al. (2007, 2011). Madhav et al. (2015) developed and validated a new set of microsatellite markers for their applicability in differentiating different types of tobacco, diverse cultivars of flue-Cured virginia (FCV) tobacco, and the transferability of these markers in a wide range of *Nicotiana* species. Cai et al. (2015) utilized the database of tobacco EST for the development of EST-SSR markers and validated them in studying the genetic differentiation among tobacco accessions.

Wang et al. (2018) detected a total of 1,224,048 non-redundant NIX (*Nicotiana* multiple (X) genome) markers (SSRs) through comparative genome wide characterization of ~20 Gb sequences from seven species viz. *N. benthamiana, N. sylvestris, N. tomentosiformis, and N. otophora,* and three *N. tabacum* cultivars (TN90, K326, and BX) (Wang et al. 2018). Such large scale development of SSR markers in tobacco has led to the analysis of molecular diversity of genetic resources (Moon et al. 2009b; Davalieva et al. 2010; Fricano et al. 2012; Gholizadeh et al. 2012; Prabhakararao et al. 2012; Xiang et al. 2017), distinctiveness uniformity stability (DUS) testing (Binbin et al. 2020), genetic relatedness of cultivated varieties (Moon et al. 2008), estimating the changes in diversity due to breeding interventions (Moon et al. 2009a) and also for the identification of linked markers and QTLs to abiotic stresses (Hatami et al. 2013).

Markers such as ISSRs (Yang et al. 2005a, 2007; Qi et al. 2006) and inter-retrotransposon amplification polymorphism (IRAP) markers (Yang et al. 2007) have also been employed to assess genetic diversity in tobacco.

Even though the application of SNPs in tobacco is complicated and challenging due to its tetraploid nature and complex genetic architecture (Ganal et al. 2009), recent studies identified number of SNPs in tobacco (Xiao et al. 2015; Thimmegowda et al. 2018; Tong et al. 2020). These SNPs are being used in characterizing of germplasm for markers linked to economically important traits including abiotic stresses, development of molecular maps, and studying genome structure and organization.

Wang et al. (2021) identified 47 core Kompetitive allele specific PCR (KASP) and 24 candidate core markers based on SNP data. KASP markers can able to discriminate between two alleles of a SNP using a common reverse primer paired with two forward primers, one specific to each allele. These core markers were utilized for the identification of tobacco varieties and fingerprinting of 216 cigar germplasm accessions.

10.5.3 Relationship with Other Cultivated Species and Wild Relatives

Cultivated tobacco belongs to the genus *Nicotiana* and family Solanaceae. Evolution and the genetic diversity in genus *Nicotiana* was studied through comparison of morphological, cytological and biochemical traits, organellar (plastid and mitochondrial) genome organization and analysis of molecular features, such as repetitive DNA sequences and the structure of various nuclear gene families (Kostoff 1943; Goodspeed 1954; Komarnitsky et al. 1998; Lim et al. 2000; Liu and Zhang 2008).

In habit and habitat, the genus *Nicotiana* resembles the two genera, *Cestrum* (8 pairs of chromosomes) and *Petunia* (7 pairs of chromosomes) (Darlington and Janaki Ammal 1945). The genus is envisaged as derived from a pre-generic reservoir of two related genera and evolving into three complexes, at the 12-paired level,

that are hypothetical precursors of the three modern sub-genera. Although 6-paired species of *Nicotiana* is not known, the predominance of 12-paired species and their compound morphological character, along with a frequency of 4–8 pairing with a mode of 6 pairs in large number of F_1 hybrids combining 12-paired species, indicates that 6 is the basic chromosome number for *Nicotiana* and both 12 and 24 are derived numbers. The 24-paired species including *N. tabacum* and *N. rustica* are modern descendants of the 12-paired progenitors entered into amphiploid origin.

Goodspeed (1954) and Goodspeed and Thompson (1959) presented the systematic classification of the genus based mainly on cytogenetic studies involving chromosome morphology, behavior in interspecific hybrids and amphiploids and aneuploids. Subsequently, additions to this classification were made by Burbidge (1960). However, in the revised systematic classification based on molecular research, subgenera were dropped retaining the division into sections (Chase et al. 2003; Clarkson et al. 2004; Knapp et al. 2004). As per the new classification, *N. trigonophylla* Dun. was renamed as *N. obtusifolia* Martens et Galeotti, *N. affinis* Hort is considered synonymous with *N. alata* Link et Otto, and *N. bigelovii* (Torrey) Watson with *N. quadrivalvis* Pursh. *N. sanderae* Hort. is considered to be hybrid between *N. alata* and *N. forgetiana* Hemsl. (*Nicotiana x sanderae*) and *N. eastii* Kostoff as an autotetraploid variant of *N. suaveolens* Lehm. (Chase et al. 2003; Knapp et al. 2004).

Among the 83 identified wild species of *Nicotiana* in the genera, *Nicotiana tabacum* and *N. rustica* are the cultivated species (Goodspeed 1954; Chase et al. 2003; Lewis 2011; Sierro et al. 2014; Berbeć and Doroszewska 2020). Both the cultivated tobacco are allopolyploid species ($2n = 4x = 48$) with basic chromosome number of $x = 12$ (Gopalachari 1984; Knapp et al. 2004). Tobacco stands out as a complex allotetraploid with a large 4.5 Gb genome with significant proportion (>70%) of repeats (Zimmerman and Goldberg 1977; Renny-Byfield et al. 2011). *N. tabacum* constitutes wide range of morphological types having diversified utilities viz., smoking, chewing, snuff, etc. Other *Nicotiana* species cultivated in smaller scale are *N. repanda* Willd ex Lehm., *N. attenuata* Torrey ex S. Watson and *N. quadrivalvis* Pursh are for smoking, *N. sylvestris* Spegazzini & Comes, *N. alata* Link and Otto, *N. langsdorffii* Weinmannm, *N. forgetiana* Hemsley, and *N. sanderae* (Hort) for ornamental and *N. glauca* Graham for industrial purpose (Lester and Hawkes 2001).

N. tabacum is natural amphidiploid (allopolyploid, $2n = 4x = 48$) ascended by hybridization of wild progenitor species, *N. sylvestris* (S-genome) x *N. tomentosiformis* (T-genome) and *N. rustica* L. from *N. paniculata*/*N. knightiana* (P/K-genome) x *N. undulata* Ruiz & Pav. (U-genome) (Goodspeed 1954; Clarkson et al. 2005; Lim et al. 2005; Leitch et al. 2008; Edwards et al. 2017; Sierro et al. 2018). Whole-genomic sequence studies indicated that the genome of *N. sylvestris* contributes 53% and *N. tomentosiformis* 47% to *N. tabacum* (Sierro et al. 2014). The comparative mapping studies suggested that the tetraploid tobacco genome has undergone a number of chromosomal rearrangements after the polyploidization (Wu et al. 2009; Gong et al. 2016). Number of reciprocal translocations and inversions (>10) has been found to differentiate the ancestral tobacco genomes from the tomato genome (Wu et al. 2009). Using cpSSRs and MtSSRs, Murad et al. (2002) concluded that the S genome

in tobacco was originated from *N. Sylvestris* ancestor. Chloroplast genome studies indicated that *N. otophora* is a sister species to *N. tomentosiformis* and *Atropha belladonna* and *Datura stramonium* are the closest relatives (Asaf et al. 2016).

Sierro et al. (2018) reported that 59% *N. rustica* genome originated from the maternal donor (*N. paniculata/N. knightiana*) and 41% from the paternal donor (*N. undulata*). Comparison of families of repetitive sequences proved that P- and U-genomes of *N. rustica* was similar to the putative parents, *N. paniculata* and *N. undulata*, respectively (Lim et al. 2005). Genomic in situ hybridization studies confirmed that *N. rustica* is an allotetraploid between *N. paniculata* (maternal P-genome donor) and *N. undulata* (paternal U-genome donor) and interlocus sequence homogenization has resulted in the replacement of *N. paniculata*-type intergenic spacer (IGS) of rDNA in *N. rustica* with *N. undulata*-type of sequence (Matyasek et al. 2003). However, analysis of nuclear genome, chloroplast genome and functional genes indicated that *N. knightiana* is more closely related to *N. rustica* than *N. paniculata.* Gene clustering revealed 14,623 ortholog groups common to other *Nicotiana* species and 207 of them are unique to *N. rustica* (Sierro et al. 2018).

Around 40% of *Nicotiana* species are allopolyploids and considered to be generated independently in six polyploidy events several million years ago (Clarkson et al. 2004; Leitch et al. 2008). Many of the diploid genome donors that make up various allopolyploid species are closely related and others are members of distantly related taxonomic sections (Clarkson et al. 2004; Leitch et al. 2008).

N. tabacum and *N. rustica* shares their basic chromosome number ($n = 12$) with other Solanaceous species such as tomato, potato, pepper and eggplant (Lim et al. 2004; Clarkson et al. 2005). Microsynteny observed at the protein level between the genomes of *N. tabacum* cv. TN90, K326 and BX and those of tomato and potato (Sierro et al. 2014).

10.5.4 Relationship with Geographical Distribution

The genus, *Nicotiana* is presumed to have had its original habitat in and around the Andes region in South America and Central America, possibly from the mild to low altitude forest margin (Goodspeed 1954). While, occurring naturally as a perennial plant, tobacco is evolved as an annual crop. Twenty of the *Nicotiana* species are native to Australia, one to Africa and 54 to North/South America (Goodspeed 1954; Burbridge 1960; Clarkson et al. 2004). *N. benthamiana*, a species indigenous to Australia, is being used extensively as a model system to study various biological processes. The cultivated species, *N. tabacum L.* (common tobacco) and *N. rustica L* are native to America and several commercial varieties of them are cultivated extensively throughout the world.

Darvishzadeh et al. (2011) reported clustering of oriental-type tobacco genotypes based on morphological traits was in agreement with their geographical distribution. The genetic diversity studies with SSR markers in oriental (Darvishzadeh et al. 2011), and RAPD and AFLP markers in flue-cured tobaccos (Zhang et al. 2008) could not

indicate any such clear pattern based on their geographical origins. However, the clustering of tobacco genotypes based on molecular diversity found to correspond to commercial classes (Flue-Cured, Burley, etc.), manufacturing trait and parentage (Sivaraju et al. 2008; Fricano et al. 2012).

10.5.5 Extent of Genetic Diversity

Existence of morphological diversity is observed in the tobacco germplasm collections maintained at tobacco gene bank in India (Baghyalakshmi et al. 2018; Sarala et al. 2018). Similarly, Moon et al. (2009b) observed large average genetic diversity among *N. tabacum* accessions from the U.S. *Nicotiana* Germplasm Collection compared to FCV tobacco accessions. While, lower SSR diversity per locus reported in similar investigations carried out on TI accessions of tobacco (Fricano et al. 2012). Low degree of genetic polymorphism was observed among tobacco cultivars by different workers (Xu et al. 1998; Del Piano et al. 2000; Rossi et al. 2001; Yang et al. 2005a; Zhang et al. 2005; Julio et al. 2006). In contrast, Xiang et al. (2017) reported the richest genetic diversity for local group of tobacco varieties and lower diversity for introductions, and higher genetic similarity values between introductions and breeding group. While, the variation among the tobacco lines for chemical traits was found to be higher (Tso et al. 1983; Darvishzadeh et al. 2011) The relatively low levels of diversity in tobacco cultivars may be due to the utilization of only small proportion of the variability of the gene pools of the progenitor species in breeding programs (Ren and Timko 2001; Lewis et al. 2007). Among the species, the level of polymorphism among the varieties of *N. tabacum* was reported to be higher compared to *N. rustica* (Sivaraju et al. 2008). However, genetic diversity among wild tobacco accessions was found to be higher (Chuanyin et al. 2009).

10.6 Association Mapping Studies

Association analysis, also known as linkage disequilibrium (LD) mapping or association mapping is based on linkage disequilibrium, which detects the frequency of significant association between the genetic variation of markers or candidate genes and target traits in the natural population (Bradbury et al. 2007; Pritchard et al. 2000). Linkage disequilibrium (LD) is defined as the non-random association of alleles at two or more loci (Fricano et al. 2012). Association analysis does not require the construction of specific mapping populations and genetic maps, thereby, considerably reduce the workload. Further, it uncovers (explores) elite genes from a certain scale (quantity) of germplasm resources in a single instance providing evidence for genetic diversity. LD mapping has advantage over traditional mapping because in a random-mating population over several generations, only close linkage between markers and traits remains, thus facilitating fine mapping.

10.6.1 Extent of Linkage Disequilibrium

Extent of linkage disequilibrium in crop plants is influenced by mutation rate, genetic drift, selection, mating system, recombination rate, gene conversion, and size and structure of the population (Flint-Garcia et al. 2003). Long- and short-range LD could be identified through high-density genome fingerprinting. In species with large genomes, a lower number of molecular markers can be tested for the identification of large LD, although this will result in a lower mapping resolution (Waugh et al. 2009). Conversely, if large panels of markers are available, short-range LD enables the fine mapping of causal polymorphisms (Myles et al. 2009).

When conducting genome-wide association studies (GWAS), the knowledge on the extent of LD is essential to estimate the minimum distance required between markers for effective coverage of the genome. Fricano et al. (2012) identified 89 tobacco genotypes that captured the whole-genetic diversity at 49 SSR loci and evaluated LD using 422 SSR markers mapped on seven linkage groups. This study clearly indicated that LD in tobacco was dependent on the population structure and extended up to a distances of 75 cM with $r2 > 0.05$ or up to 1 cM with $r2 > 0.2$.

10.6.2 Target Gene Based LD Studies

LD has been used locate QTLs or major genes, based on the co-segregation of specific marker alleles and traits in tobacco (Zhu et al. 2008; Rafalski 2010). Zhang et al. (2012) conducted association analysis and detected 18 sequence-related amplified polymorphism markers significantly associated with six agronomic traits in 258 flue-cured tobaccos. One SSR marker and six microsatellite-anchored fragment length polymorphism markers found to be associated with the levels of tobacco-specific nitrosamines (Yu et al. 2014). Twenty four SSR loci associated with aroma substances in tobacco (Ren et al. 2014) and one SSR locus from linkage group 13 with low chloride accumulation rate in 70 oriental-type tobaccos (Basirnia et al. 2014). Fan et al. (2015) performed a marker–trait association analysis and obtained 11 SSR markers associated with potassium content in tobacco; five among the 11 SSR markers were selected to validate the stability of the associated markers by scanning 130 other tobacco germplasms. Tong et al. (2020) made association analysis of leaf chemistry traits in natural populations using a large number of tobacco germplasms based on genome-wide SNPs.

10.6.3 Genome Wide LD Studies

Genome wide LD studies are not reported in tobacco as on date. Association mapping studies largely depends on population genetic structure. Based on the existing molecular diversity in germplasm collections, the population structure could be reconstructed in tobacco for association studies (Moon et al. 2009b). Ganesh et al. (2014) observed that 25 unlinked SSR markers delineated genetic structure of 135 FCV (fluecured virginia) tobacco genotypes revealing a total of 85 alleles with an average of 3.4 alleles per locus.

10.6.4 Future Potential for the Application of Association Studies for Germplasm Enhancement

The population-based association studies utilizes the available broader genetic variations in wider background and detects marker-trait correlations. Hence, they can lead to construction of higher resolution maps with broader allele coverage because of the utilization of majority recombination events from a large number of meiosis throughout the germplasm development and exploits historically measured trait data without the development of expensive and tedious biparental populations saving time and costs involved (Abdurakhmonov and Abdukarimov 2008). LD based association studies provide an opportunity to dissect and exploit existing natural variations in tobacco germplasm resources for tobacco improvement. Availability of large collection of germplasm resources in tobacco over worldwide facilitate to detect neutrally inherited markers linked to genetic causatives or genes controlling the complex quantitative target traits including resistance to abiotic stresses.

10.7 Brief Account of Molecular Mapping of Resistance Genes and QTLs

10.7.1 Brief History of Mapping Efforts

The identification of linkage between pollen color factor and the sterility factors in tobacco, initiated gene mapping research in tobacco (Anderson and Dorothea 1931; East 1932; Brieger 1935; Smith 1937). Establishing two types of monosmics, Clausen and Goodspeed (1926) demonstrated haplo-C (then called “corrugated”) is involved in the chromosome in which the basic color factor, Wh, is located. Later, the association in transmission between 24 monosomics developed by Clausen and Cameron (1944) led to location of 18 genes in nine chromosomes. Though genes regulating various traits and their linkages with other genes are identified, detailed

map based entirely on genes is not available in tobacco (Suen et al. 1997; Narayanan et al. 2003). Efforts were initiated during early nineties to map and tag resistant genes linked to various stresses with DNA markers such as RFLP, RAPD and AFLPs. Initially, RFLP and RAPD markers were used to map *Nicotiana* spp. (Lin et al. 2001) Later, RAPD, AFLPs and ISSR were used in construction of genetic maps (Lin et al. 2001; Nishi et al. 2003; Julio et al. 2006; Xiao et al. 2006). With the sighting of SSR markers in late 1990s, SSR based molecular map showing 24 linkage groups was developed in *N. tabacum* (Bindler et al. 2007). This SSR map was improved further with identification of a more number of SSRs (Bindler et al. 2011; Tong et al. 2012). With the identification of SNPs in recent years, high density SNP based tobacco genetic map has been developed with 24 linkage groups (Tong et al. 2020). Currently, maps are available for selected *Nicotiana* spp. FCV and burley tobacco types (see Sect. 10.7.5).

10.7.2 Evolution of Marker Types: RFLPs to SNPs

Variations existing among individuals for specific regions of DNA are deducted by molecular markers and hence serves as useful tools in mapping of genetic material. Molecular genetic markers, such as RFLP, RAPD, AFLP, microsatellites or SSRs, and SNPs have been used in genetic linkage mapping and QTL mapping in tobacco (Liu and Zhang 2008). In the initial stage, PCR-based RAPD markers were used by different researchers to map and tag resistant genes linked to abiotic stresses due to their relative ease in spite of the reproducibility issues. Although, reproducibility and sensitivity of AFLPs markers is higher, they were used in a limited degree in mapping of resistance genes due to their lengthy and laborious detection method, low reproducibility and non-suitability to automation. In late 1990s with the discovery of SSR markers, SSRs and EST-SSRs became markers of choice for mapping in tobacco (Bindler et al. 2007, 2011; Tong et al. 2012). Currently, more than 10,000 SSR markers are available in tobacco for their use in QTL/gene mapping studies (Bindler et al. 2007, 2011; Tong et al. 2012; Cai et al. 2015; Madhav et al. 2015). In addition, Wang et al. (2018) identified a huge number of about 1,200,000 non-redundant and novel NIX (*Nicotiana* multiple (X) genome) markers (SSRs) for use in tobacco.

With the advent of SNPs in recent past, mapping of tobacco genome using these markers proved an easy platform for mapping. Xiao et al. (2015) developed SNPs using two different methods (with and without a reference genome) based on restriction-set associated DNA sequencing (RAD-seq). Through whole-genome resequencing of 18 FCV tobacco genotypes, Thimmegowda et al. (2018) identified and positioned SNPs into linkage groups. Using *N. tabacum* (K326 cultivar) as a genome reference, Tong et al. (2020) identified and mapped 45,081 SNPs to 24 linkage groups on the tobacco genetic map. Adding advantage to the above markers others such as; sequence-specific amplification polymorphism (SSAP), sequence-related amplified polymorphism (SRAP), cleaved amplified polymorphic sequence

(CAPS) and diversity arrays technology (DarT) were also used in molecular mapping in tobacco.

10.7.3 Mapping Populations Used

Diverse populations viz., F_2 populations, doubled haploid (DH) lines, recombinant inbred lines (RILs), BC_1 progenies, BC_1F_1, BC_4F_3 populations etc. have been used as the for molecular mapping in tobacco (Table 10.1). Majority of the maps developed were based on F_2 and DH populations and other maps were developed based on next-generation sequencing (NGS) technologies. Practically, the population size need to be around 99–381 individuals in a mapping population for higher resolution and fine mapping.

10.7.4 Mapping Software Used

In developing molecular maps of tobacco, softwares such as Mapmaker program (Lander et al. 1987; Lin et al. 2001; Wu et al. 2010), JoinMap® 3.0 program (Van Ooijen and Voorrips 2001; Bindler et al. 2007, 2011), Map Manager QTXb20 (Manly et al. 2001; Bindler et al. 2011; http://www.mapmanager.org) and JoinMap 4.0 (Van Ooijen 2006; Lu et al. 2012; Tong et al. 2016) and LepMap3 software (Rastas 2017; Tong et al. 2020, 2021) were used. Among the softwares, JoinMap 3.0/4.0 program is the widely used one for construction of molecular maps in tobacco. Similarly, LepMap3 software was used in the building of maps using NGS data.

10.7.5 Maps of Different Generations

For studies of genetics, genomic structure, genomic evolution and for mapping essential traits, genetic linkage maps are vital. In case of tobacco, genetic map construction has lagged behind other *Solanaceae* crops such as the tomato, potato, and pepper plants (Barchi et al. 2007; Jacobs et al. 2004; Tanksley et al. 1992). Till the end of twentieth century, scanty data was available on genetic mapping and molecular marker development in tobacco (Suen et al. 1997). Construction of genetic linkage maps in tobacco was started at the beginning of twenty-first century (Lin et al. 2001). Various maps constructed are briefly discussed here.

Table 10.1 Molecular linkage maps constructed in Nicotiana

S. No.	Population	Markers used	Linkage groups identified	Total length covered (cM)	References
A	**Nicotiana species**				
1	99 F_2 plants from the cross *N. plumbaginifolia* × *N. longiflora*	69 RFLP and 102 RAPD	9	1,062	Lin et al. (2001)
2	*N. tomentosiformis*	489 SSRs/CAPS	12	~1,000 cM	Wu et al. (2010)
3	*N. acuminata*	308 SSRs/CAPS	12	~1,000 cM	Wu et al. (2010)
B	**Burley**				
1	125 DH lines, derived from F_1 hybrids between *W6* and *Michinoku 1*	117 AFLPs	10	383	Nishi et al. (2003)
2	92 DH population derived from Burley 37 and Burley 21	112 AFLP loci and 6 SRAP loci	22	1,953.6 cM	Cai et al. (2009)
C	**Flue-cured**				
1	DH population from a cross between Speight G-28 and NC2326	11 ISSRs and 158 RAPDs	27	2,094.6	Xiao et al. (2006)
2	114 recombinant inbred lines	138 ISSR, AFLP and SSAP markers	18	707.6	Julio et al. (2006)
3	186 F_2 derived from Hicks Broad Leaf and Red Russian	293 SSRs	24	1,920	Bindler et al. (2007)
4	186 F_2 derived from cultivar, Hicks Broad Leaf and Red Russian	2318 SSRs	24	3,270	Bindler et al. (2011)
5	207 DH population derived from FCV cultivars, HHDJY and Hicks Broad Leaf	851 (238 DArT and 613 SSRs)	24	2,291	Lu et al. (2012)

(continued)

Table 10.1 (continued)

S. No.	Population	Markers used	Linkage groups identified	Total length covered (cM)	References
6	Parents [HD (Honghua Dajinyuan) and RBST (Resistance to Black Shank Tobacco)] and their F_1 (HD × RBST) and 193 BC_1 progenies	Map 1: 4138 SNPs and Map 2: 2162 SNPs	24	Map 1: 1,944.74 (with reference genome); Map 2: 2,000.9 (de novo identification SNPs by RAD-seq)	Xiao et al. (2015)
7	Specific locus-amplified fragment sequencing	4215 SNPs and 194 SSRs	24	2,662.43	Gong et al. (2016)
8	213 BC_1s derived from Y3 and K326	626 SSRs	24	1,120.45	Tong et al. (2016)
9	Parents (Y3 and K326), their F_1 and 271 RILs	45,081 SNPs	24	3,486.78 cM	Tong et al. (2020)
10	Parents (NC82 and TT8), their F_1 and 200 BC_1F_1 s	13,273 SNPs	24	3,421.80	Cheng et al. (2019)
11	Parents (Y3 and K326), their F_1 and 381 $BC4F_3$s	24,142 SNPs	24	2,885.36	Tong et al. (2021)
D	**Inter type**				
1	187 F_2 derived from the cross Taiyan 7 (FCV) and Bailei 21 (burley)	112 (92 SRAP and 10 ISSR)	26	1,560.2	Ma et al. (2008)

Note RFLP—Restriction fragment length polymorphisms; RAPD—Random amplified polymorphic DNA; AFLP—Amplified fragment length polymorphism; ISSR—Inter simple sequence repeat; SSAP—Sequence-specific amplification polymorphisms; SSR—Simple sequence repeats; SRAP—Sequence-related amplified polymorphism; CAPS—Cleaved Amplified Polymorphic Sequences; DArT—Diversity arrays technology; and SNP—single nucleotide polymorphisms; DH—Doubled haploids; RILs—Recombinant inbred lines; FCV—Flue-cured virginia

10.7.5.1 Mapping of *Nicotiana* Species

Lin et al. (2001) constructed a genetic linkage map based the F_2 plants (99 individuals) derived from tobacco wild species, *Nicotiana plumbaginifolia* × *N. longiflora.* This map covers 60 RFLP and 59 RAPD loci spread on nine major linkage groups measuring 1,062 cM. The tenth linkage group could not be identified due to unavailability of markers, corresponding to the haploid chromosome number of

N. plumbaginifolia. Wu et al. (2010) generated two maps for wild diploid *Nicotiana* species, *N. tomentosiformis* and *N. acuminata* with 12 linkage groups spanning ~1,000 cM. A combination of 489 SSR and cleaved amplified polymorphic sequence (CAPS) markers was used to construct *N. tomentosiformis* map constructed and, while the *N. acuminata* (closely related to *N. sylvestris*) map was generated with a mixture of 308 SSR and CAPS markers (Wu et al. 2010).

10.7.5.2 Mapping of Burley Tobacco

A genetic map of the burley tobacco was constructed using AFLP based on DH lines with 10 linkage groups was derived from F_1 hybrids between burley entries, W6 and Michinoku 1 (Nishi et al. 2003). The currently available high density burley linkage map was generated assembling 112 AFLP loci and six SRAP loci into 22 linkage groups (A1-A22) covering ~1,954 cM using a DH population derived from a cross between Burley 37 (high nicotine content) and Burley 21 (low nicotine content) (Cai et al. 2009).

10.7.5.3 Mapping of Flue-Cured Tobacco

The first linkage map of flue-cured tobacco based on a DH population was developed from a cross between Speight G-28 and NC2326 (Xiao et al. 2006) using 169 ISSR/RAPD molecular markers covering 27 linkage groups. While a molecular linkage map of flue-cured tobacco with 18 linkage groups covering 138 ISSR, AFLP and SSAP markers based on 114 flue-cured tobacco RILs was constructed by Julio et al. (2006).

Bindler et al. (2007, 2011) constructed an enriched SSR based linkage map with 2,318 SSR markers covering 24 linkage groups with a total length of 3,270 cM using an F_2 population from a cross between Hicks Broadleaf × Red Russian. This map is the most widely used map of tobacco and the average genetic distance between the markers was 1.4 cM. There still exists some gaps of about ~16 cM in this map, in spite of the high-density marker used (Fig. 10.1; Bindler et al. 2011). Tong et al. (2012) used double haploid (DH) lines derived from a cross between 'Honghua Dajinyuan' and 'Hicks Broad Leaf' with 207 individuals and constructed a genetic map of flue-cured tobacco consisting of 611 SSR loci distributed on 24 tentative linkage groups covering a total length of ~1,882 cM. Tong et al. (2016) constructed a genetic map entailing 626 SSR loci distributed across 24 linkage groups covering a total length of about 1,120 cM utilizing 213 backcross (BC1) individuals derived from an intra-type cross between two flue-cured tobacco varieties, Y3 and K326.

Xiao et al. (2015) constructed two linkage maps with a total of 2,162 and 4,138 SNP markers covering around 2,001 and 1,945 cM, into 24 different linkage groups based on reference genome and without reference, respectively. SOL Genome Network released SNP-based high density genetic map, *N. tabacum* 30 k Infinium HD

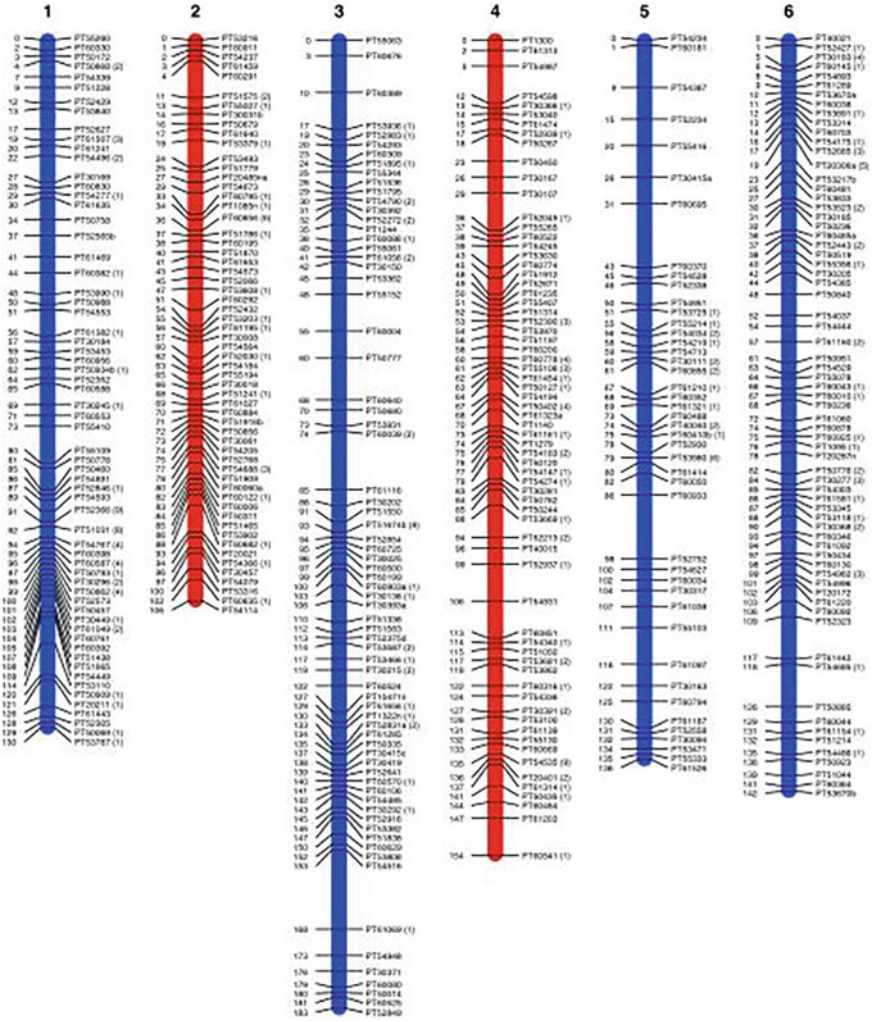

Fig. 10.1 Part SSR map (1–6 linkage groups) constructed by Bindler et al. (2011) with 2,318 microsatellite markers covering a total length of 3,270 cM

consensus map 2015 (https://solgenomics.net/cview/map.pl?map_version_id=178). With restriction site-associated DNA sequencing, Cheng et al. (2019) constructed a high-density SNP genetic map of flue-cured tobacco using restriction site-associated Illumina DNA sequencing. In this map, a total 13,273 SNP markers were mapped on 24 high-density tobacco genetic linkage groups spanning around 3,422 cM, with a mean distance of 0.26 cM between adjacent markers. Tong et al. (2020) identified a total of 45,081 SNP markers (with 7,038 bin markers) and characterized to construct a high-density SNP genetic map of flue-cured tobacco spanning a genetic distance

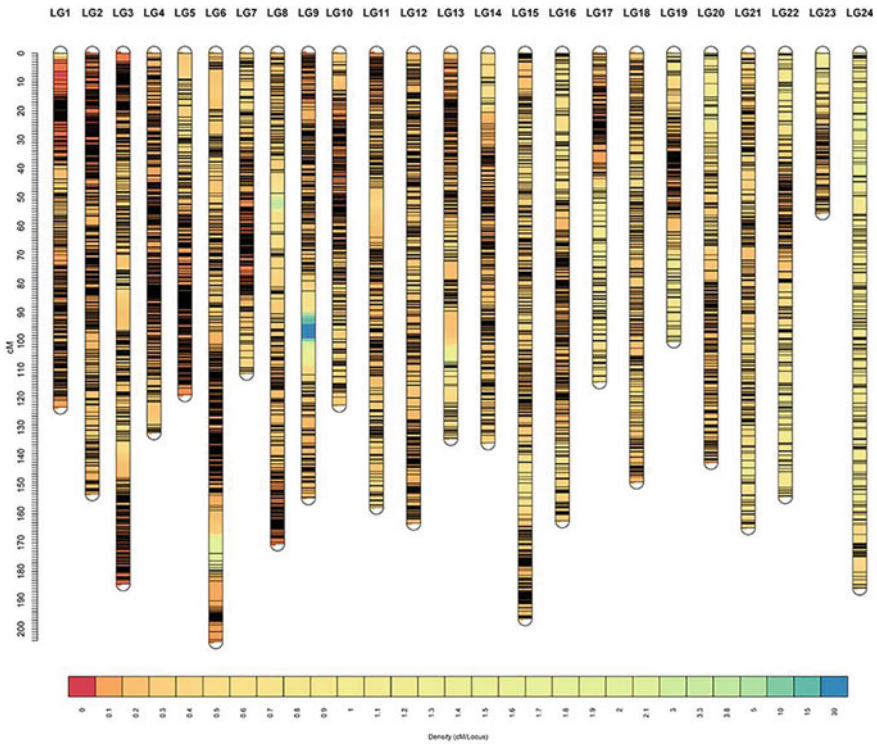

Fig. 10.2 Linkage map constructed by Tong et al. (2020) covering a total length of 3486.78 cM

of 3,487 cM (Fig. 10.2). Tong et al. (2021) successfully constructed another high-density genetic map with 24,142 SNP markers using a BC_4F_3 population derived from inbred of flue-cured tobacco lines Y3 (recurrent parent) and K326 (donor parent). This map included 4,895 bin markers with a genetic distance of ~2,886 cM and an average genetic distance of 0.59 cM.

Lu et al. (2012) developed a high-density integrated linkage map (2,291 cM) of flue-cured tobacco that included 851 markers [238 diversity arrays technology (DarT) and 613 SSR] in 24 linkage groups. Gong et al. (2016) generated a high-density ~2,662 cM length integrated genetic map of flue-cured tobacco containing 4,215 SNPs and 194 SSRs distributed on 24 linkage groups with an average distance of 0.60 cM between adjacent markers.

10.7.5.4 Intra Type Genetic Maps

Ma et al. (2008) constructed an intra type genetic map of flue-cured and burley tobaccos, based on sequence related amplified polymorphisms (SRAPs) and ISSR markers, containing 26 linkage groups and 112 markers. Currently, the available

high-density maps in tobacco are constructed with SSR (Bindler et al. 2011) and SNP (Gong et al. 2016; Tong et al. 2020) markers as detailed above. The widely referred SSR map of Bindler et al. (2011) was constructed with 2,318 microsatellite markers covering a total length of 3,270 cM while the SNP map of Tong et al. (2020) covers ~3,487 cM with 45,081 SNPs. The combination of SNPs and genetic maps, if developed, helps in designing precise breeding strategies and genomic selection in tobacco. Diverse genetic maps existing at present constructed can be effectively utilized in mapping QTLs, positional cloning, comparative genomics analysis, marker-assisted breeding and genomic selection etc. It would be necessary to further build the genetic linkage maps of tobacco in different cultivating types for their effective utilization in breeding of those types.

10.7.6 Enumeration of Mapping of Simply-Inherited Stress Related Traits

The availability of *Nicotiana* genome sequences (Sierro et al. 2014; Edwards et al. 2017) information and high-density molecular maps in recent times is laying the foundation for trait discovery and fine mapping of trait of interest in tobacco (Yang et al. 2019). Tobacco plant cope with abiotic stresses through activation and regulation of specific stress-related genes. The genes involved in the whole-sequence of molecular responses to abiotic stresses include genes for signaling, transcriptional control, protection of membranes and proteins, and free-radical and toxic-compound scavenging (Wang et al. 2003; Xiang et al. 2016; Yang et al. 2016). Hence, resistance or tolerance to a specific stress is not controlled by a single gene. In general, abiotic stress are controlled by poly genes with low heritability and are influenced by environment. In view of these complications the studies showing mapping of abiotic stress tolerance in tobacco is scanty.

10.7.7 Framework Maps and Markers for Mapping Resistance QTLs

Framework maps constructed using SSR and SNP markers that were already identified and mapped to linkage groups in tobacco (Lu et al. 2012; Tong et al. 2020). High density SSR map of Bindler et al. (2011) and SNP map of Tong et al. (2020) can be the ideal ones for constructing framework maps while mapping various traits (Edwards et al. 2017). The SNP-based high density genetic map, *N. tabacum* 30 k Infinium HD consensus map 2015 can be one of the best resources for fine mapping any trait of interest (https://solgenomics.net/cview/map.pl?map_version_id=178).

In few cases for reliability and consistency RAPD markers are being converted into sequence characterized amplified region (SCAR) markers. SCAR markers can be

developed after sequencing the resultant RAPD bands and designing 18–25 base PCR primers that can specifically amplify the sequenced DNA segment. CAPS, conserved ortholog sequences (COS), random amplified microsatellite polymorphism (RAMP), ISSRs and target region amplification polymorphism (TRAP) are some of the other markers that can be used in map construction. CAPS markers developed are the primers designed on the known sequence of a gene of interest. COS primers used are universal primers based on sequence alignments of orthologs (genes that are conserved in sequence and copy number) from multiple solanaceous species. RAMP markers include SSR primers that amplify the genomic DNA in the presence or absence of RAPD primers. TRAPs are two PCR-based primers, one from target EST and the other is an arbitrary primer.

10.7.8 QTL Mapping Software Used

Mapmaker/Exp 3.0 is the widely used mapping software in mapping of various QTL traits in tobacco followed by various version of Join Map and Map Chart. Some of the other softwares used are Mapmaker/QTL, QTL IciMapping 4.1, QTL Network 2.1, R/QTL, AYMY-SS, Stat Graphics Plus 5.0 and QTL Cartographer V 2.5 (Sarala et al. 2021).

10.7.9 Details on Trait Wise QTLs

Biparental mapping populations have been used to identify QTLs controlling chloride accumulation rate in tobacco genome (Li-Hua et al. 2011; Hatami et al. 2013). Using family-based linkage mapping approach, Li-Hua et al. (2011) detected two QTLs for total chlorine concentration. Hatami et al. (2013) developed a genetic linkage map for oriental-type tobacco by using F_2 generation individuals from the cross between two divergent oriental-type tobacco genotypes viz., 'Basma Series 31' and 'SPT 406'. Through single marker analysis of the above F_2 population, SSR marker PT30346 was found to be significantly associated with chloride accumulation. They have further identified two QTLs for chloride accumulation in the leaf, namely, Chl_{IM} (on LG II) and Chl_{CIM} (on LG V) with R^2 values (phenotypic variance) of 0.4 and 0.07, using interval mapping (IM) and composite interval mapping (CIM), respectively (Fig. 10.3). A LOD score of 2.3 was used for identifying these significant QTLs that were estimated to originate from maternal parent, Basma Series 31.

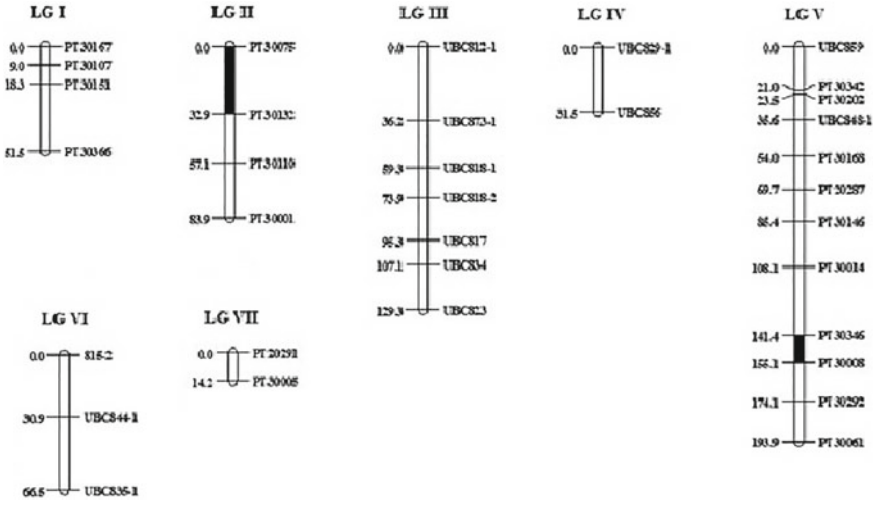

Fig. 10.3 Linkage map of SSR and ISSR markers in oriental tobacco showing the location of QTLs affecting chlorine accumulation. Bars represent intervals associated with the QTLs (Hatami et al. 2013)

10.8 Marker-Assisted Breeding for Resistance Traits

Marker-assisted breeding (MAB) refers to the breeding procedure in which DNA marker detection and selection are integrated into a traditional breeding programs. The status of MAB and its prospects are discussed here. The advantage of using DNA markers is that they can be detected at any stage of plant growth in contrast to that of classical markers detection of which is usually limited to certain growth stages. Polymorphism for DNA markers is available throughout the genome, and their presence or absence is not affected by environments and usually do not directly affect the phenotype. If the markers are located in close proximity to the target gene or present within the gene, selection of the markers will ensure the success in selection of the gene. Thus, DNA markers are the major types of genetic markers for MAB (Jiang 2013). Most commonly used molecular markers in tobacco include RAPD, AFLP, SSR, SCAR, CAPS, dCAPS (derived CAPS), and KASP (Yang et al. 2019). Different DNA markers have own advantages and disadvantages for specific purposes. Comparatively, SSRs have most of the desirable features and availability of large number of SSRs make them markers of choice in tobacco. SNPs require more detailed knowledge of the specific, single nucleotide DNA changes responsible for genetic variation among individuals. Fairly large number of SNPs have become available in tobacco making them important choice of markers for MAB in tobacco.

10.8.1 Germplasm Characterization and DUS

Initiation of marker-assisted breeding program requires the identification of closely linked markers for target traits in germplasm. These tightly linked markers can be utilized in MAB while screening parents, F_1 and other segregating materials for selecting plants with target traits. Gene mapping, QTL analysis, association mapping, classical mutant analysis, linkage or recombination analysis, bulked segregant analysis, etc. provides information on marker trait associations in germplasm lines and mapping populations. It is also important to identify the linkage situation i.e. cis/trans (coupling or repulsion) linkage with the desired allele of the trait.

Large number of studies for screening germplasm and sources of resistance are made in tobacco to identify closely linked markers to various abiotic stresses for their introgression into cultivated varieties. QTL analysis and genetic mapping, through biparental or association mapping (AM) populations, have accelerated the dissection of genetic control of stress resistant traits in tobacco. Tightly linked markers are important tools for DUS characterization of varieties also. This information has the potential to make MAS a successful option for tobacco improvement.

10.8.2 Marker-Assisted Gene Introgression

Marker-assisted back crossing (MABC) is the simplest form of resistant trait introgression that is most widely and successfully used in transferring abiotic stress resistant genes/QTLs into elite cultivars. MABC aims to transfer one or a few genes/QTLs for resistance from one genetic source (donor parent) into a superior cultivar or elite breeding line (recurrent parent) to improve the stress resistance. Unlike traditional backcrossing, MABC is based on the alleles of markers associated with or linked to gene(s)/QTL(s) of interest instead of phenotypic performance of target trait. MABC program with foreground selection for the marker allele(s) of donor parent at the target locus (ex. resistance) ensures the transfer of target trait from donor parent. Background selection for the marker alleles of recurrent parent in all genomic regions of desirable traits (agronomic traits) except the target locus will takes care of the genome recovery of recurrent parent while effectively transferring of resistance into elite genotypes (Hospital 2003). MAS can be used when other characters are to be combined from two parents along with resistance trait. However, MAS will be highly effective for simply inherited character controlled by a few genes than for a complex character governed by large number of genes.

10.8.3 Gene Pyramiding

Pyramiding of several genes/QTLs can be achieved through multiple-parent crossing or complex crossing, multiple backcrossing, and recurrent selection. A number of factors are involved in deciding suitable breeding scheme for marker-assisted gene pyramiding such as the number of parents that contain the desired genes/QTLs, number of genes/QTLs required to be transferred, the heritability of traits of interest, and other factors (e.g. marker-gene association and genotyping costs). Pyramiding of three or four desired genes/QTLs exist in three or four different lines can be realized by three-way, four-way or double crossing. They can also be brought together by convergent backcrossing or stepwise backcrossing. Complex or multiple crossing and/or recurrent selection may often be preferred for pyramiding of more than four genes/QTLs.

Gene pyramiding can be achieved through three different strategies or breeding schemes namely stepwise, simultaneous/synchronized and convergent backcrossing or transfer. In the stepwise backcrossing, the target genes/QTLs are transferred from donor parents one after the other into the recurrent parent (RP). Gene pyramiding through stepwise backcrossing is easier and more precise to adopt as it transfer only one gene/QTL at a time requiring small population size and lower genotyping cost. But this method takes a longer time to complete. In the simultaneous (synchronized) backcrossing, the recurrent parent is initially crossed to each of the donor parents and the resultant single-cross F_1s are crossed with each other to produce two double-cross F_1s and then the two double-cross F_1s are crossed to produce a hybrid integrating all the target genes/QTLs in heterozygous state. This hybrid and its progeny with heterozygous markers for the target genes/QTLs is subsequently back crossed to the RP until the satisfactory recovery of the RP genome. Finally, the RP genome recovered line is selfed to achieve homozygosity. Simultaneous or synchronized backcrossing requires a large population and more number of genotypings as all target genes/QTLs are handled at the same time and takes shorter time to transfer multiple genes. Both stepwise and synchronized backcrossing strategies are employed in convergent backcrossing. First each of the target gene/QTLs from the donors are transferred separately into the RP through single crossing followed by backcrossing based on the linked markers to produce improved lines. The improved lines are crossed with each other and the consequential hybrids are intercrossed to pyramid all the genes/QTLs into the final improved line. Convergent backcrossing require less time (compared to stepwise transfer) and easily fix and pyramid genes (compared to simultaneous transfer).

If all the parents are improved cultivars with complementary genes or favorable alleles for the traits of interest, marker-assisted complex or convergent crossing (MACC) can be undertaken to pyramid multiple genes/QTLs. In this method, the hybrid of convergent crossing is selfed and MAS for target traits is performed for several consecutive generations until genetically stable lines with desired marker alleles and traits are achieved. For reducing the population size and avoiding the loss of most important genes/QTLs, the most important genes/QTLs can be detected and

selected first in early generations and less important markers later. Theoretically, it is possible to apply MABC and MACC for pyramiding target genes/QTLs in tobacco crop. Currently information is not available about the release of commercial varieties developed using these strategies.

10.8.4 Limitations and Prospects of MAS and MABCB

MAS and marker-assisted back cross (MABC) breeding may not be universally useful in spite of their advantages (Jiang 2013). Quick DNA extraction techniques and a high throughput marker detection system are essential to handle large number of samples and large-scale screening of multiple markers. Development of suitable bioinformatics and statistical software packages are required for efficient and quick labeling, storing, retrieving, processing and analyzing large data set requirements, and even for integrating data sets available from other programs. Hence, the startup expenses and labor costs involved in MAS and MABCB are higher compared to conventional techniques making them not in the reach of all the researchers (Morris et al. 2003).

When the distance between the marker and the gene of interest is higher, the chance of recombination between gene and marker increases there-by make the selection of resistant plants based on marker ineffective due to false positives. Use of flanking markers on either side of the locus of interest will increase the probability that the desired gene is selected. Sometimes markers that were used to detect a locus may not be 'breeder-friendly'. Such markers viz., RFLP and RAPD may need to be converted into more reliable and easier to use markers. RFLP markers may be converted to STS (sequence tagged site) for detection via PCR protocols (Ribaut and Hoisington 1998) and RAPD markers into SCAR markers for reliable and repeatable amplifications (Lewis 2005; Milla et al. 2005). Inaccurate estimates of locations and effects of QTLs may result in slower progress than expected through MAS (Beavis 1998). Yet times, markers developed for MAS in one population may not be suitable for screening other populations, either due to lack of marker polymorphism or the absence of a marker-trait association.

With the increasing utilization of molecular markers in various fields viz., germplasm evaluation, genetic mapping, map-based gene discovery, characterization of traits etc., MAB is going to become a powerful and reliable tool in genetic manipulation of important traits in tobacco. Availability of high density linkage maps in tobacco provides a framework for identifying marker-trait associations and selecting markers for MAB. Markers linked to resistant traits can fruitfully be utilized in MAB in tobacco. Only the markers that are closely associated with the target traits or tightly linked to the gene of interest can provide sufficient guarantee for the success in practical breeding. Availability of new high-throughput marker genotyping platforms for the detection of SSR and SNP markers along with the sequencing information of cultivated and wild relatives of *Nicotiana* is going to have a great impact on discovering marker trait associations that can be used for MAS in the future. Array-based

methods such as DArT (Lu et al. 2012) and single feature polymorphism (SFP) detection (Rostoks et al. 2005) offer low-cost marker technologies for whole-genome scans in tobacco. Rapid growth in genomics research and huge data generated from functional genomics in tobacco in the recent years is leading to the identification of many candidate genes for numerous traits including abiotic stress resistance. SNPs within candidate genes could be extremely useful for 'association mapping' and circumvents the requirement for constructing linkage maps and performing QTL analysis for new genotypes that have not been previously mapped. The availability of large numbers of publicly available markers and the parallel development of user-friendly databases (Sol genome network, NCBI etc.) for the storage of marker and QTL data, increasing number of studies on genes and marker trait associations will undoubtedly encourage the more widespread use of MAS in tobacco.

Closely linked markers allows the selection of disease/pest resistance traits even without the incidence of pests and diseases. MAS based on markers tightly linked to the multiple genes/QTLs for traits of interest can be more effective in pyramiding desirable genes than conventional breeding. Selection for all kinds of traits at seedling stage in MAB helps to minimize the costs as undesirable genotypes are eliminated at early stages. Use of co-dominant markers (e.g. SSR and SNP) in MAB allow effective selection of recessive alleles in the heterozygous state without selfing or test crossing, thus saves time and accelerate breeding progress. As more and more newer techniques are available genotypic assays based on molecular markers may be faster, cheaper and more accurate than conventional phenotypic assays and thus MAB may result in higher effectiveness and higher efficiency in terms of time, resources and efforts saved in future.

MAB has brought great challenges, opportunities and prospects for breeding crops including tobacco. As a new member of the whole family of plant breeding, MAB cannot replace conventional breeding, but can be a supplementary addition to conventional breeding. Higher costs and technical demands of MAB will continue to be an obstacle for its large-scale use, especially in the developing countries (Collard and Mackill 2008). Integration of MAB into conventional breeding programs will be an optimistic strategy for tobacco improvement in the future.

10.9 Map-Based Cloning of Resistance Genes

10.9.1 Traits and Genes

Identification and subsequent mapping of interesting mutants became difficult in view of the high levels of redundancy between genes in the large and complex genome of tobacco with the absence of molecular markers and genomic resources till recent years. Having anchored 64% of the genome assembly to chromosomal locations in recent years, a possibility now exists for map-based cloning of abiotic stress resistant genes (Edwards et al. 2017). For the first time, successful map-based cloning in

tobacco was done by Edwards et al. (2017) for NtEGY1 and NtEGY2 homeologous candidate genes for YB1 and YB2 loci conferring white stem phenotype in recessive condition in burley tobacco.

10.9.2 Strategies: Chromosome Landing and Walking

Currently available high density genetic maps, genome sequences and bacterial artificial chromosome (BAC) clones are paving the way for map based cloning of resistance genes in tobacco. In general, chromosome landing and walking strategies are used in identification of clones carrying gene of interest for map based cloning. However, in the only reported case of map based cloning in tobacco, Edwards et al. (2017) used a specific technique to clone genotyped pairs of near-isogenic lines (NILs) carrying dominant or recessive alleles of the *YB1* and *YB2* loci (cultivars SC58, NC95, and Coker 1) with a custom 30 K Infinium iSelect HD Bead Chip SNP chip (Illumina Inc., San Diego, CA) that was used in developing a high density genetic map (*N. tabacum* 30 k Infinium HD consensus map 2015; https://solgenomics.net/cview/map.pl?map_version_id=178). Genomic regions comprising SNP polymorphisms distinguishing the nearly isogenic lines were identified and SNP markers closely linked to the loci were aligned to the genome assembly and predicted potential candidate genes. Coding regions of candidate genes were then amplified, using the primers specifically designed, from first-strand cDNA from tobacco cultivars K326 and TN90. The fragments, thus amplified were finally cloned into a vector.

10.9.3 Libraries

Physical mapping, comparative genome analysis, molecular cytogenetics etc. requires the availability of high-capacity libraries. Such resources are also powerful tools for large-scale gene discovery, elucidation of gene function and regulation, and map-based cloning of target trait loci or genes associated with important agronomic and resistant traits and use in crop improvement programs. BAC libraries are the large DNA insert libraries (inserts of DNA up to 200,000 base pairs) of choice for genomics research. Cloning of larger DNA segments (more than 1000 kb) are possible with yeast artificial chromosome (YAC) libraries and greatly facilitates chromosome walking and physical mapping around the target locus. While, transformation-competent artificial chromosome (TAC) libraries make it possible to clone and transfer genes efficiently into plants. In recent years, BAC libraries are constructed and utilized in tobacco for genome sequencing, mapping and comparative genome analysis. Reports currently not available regarding the construction of YAC and TAC libraries in tobacco.

Tobacco Genome Initiative (TGI) generated a BAC library (9.7-fold genome coverage) for assembling the partial genome of Hicks Broad leaf variety and used

425, 088 BAC clone library for construction of physical map and ancestral annotation of tobacco cultivar, Hicks Broadleaf (Opperman et al. 2003; Rushton et al. 2008; Sierro et al. 2013b). Edwards et al. (2017) constructed two libraries having 150,528 BACs from K 326 variety using HindIII or EcoRI, with average insert sizes of 115 kb and 135 kb, respectively (representing ~8 × coverage of the genome) for generating a whole-genome profile (WGP) map. Jingjing (2018) reported a tobacco genome sequence of the HongDa cultivar using the combination of BAC-to-BAC libraries and whole-genome shotgun technologies. Yuhe (2012) constructed a BAC library of wild tobacco, *N. tomentosiformis* (one of the parent of *N. tabacum*) with the average DNA inserted size of 110 kb.

10.9.4 Test for Expression

The function of a target gene can successfully be validated through the transformation of a cloned gene into mutant plant and looking for wild phenotype rescue. As on date, mutant complementation studies with cloned genes are not reported in tobacco in view of the absence of map based cloning of functional genes in general and abiotic stress resistant genes particular.

10.10 Genomics-Aided Breeding for Resistance Traits

10.10.1 Structural and Functional Genomic Resources Developed

Structural genomics deals with the structure of the genome and the knowledge on genomic structure is essential for gene tagging, identification and cloning of novel genes for further genomic assisted breeding. The genome sequencing information of 12 *Nicotiana* species (*N. tabacum, N. rustica, N. attenuata, N. benthamiana, N. knightiana, N. obtusifolia, N. otophora, N. paniculata, N. sylvestris, N. tomentosiformis, N. undulata* and *N. glauca*) are available at NCBI website (https://www.ncbi.nlm.nih.gov/) and Sol Genome Network (SGN) (Asaf et al. 2016).

Advances in transcriptomic analysis and functional genomics in tobacco led to development of large data sets and tools. A data base of well characterized 2,513 transcriptions factors (TFs) was developed in tobacco using a dataset of 1,159,022 gene-space sequence reads (Rushton et al. 2008). Further, the transcriptional activity for thousands of tobacco genes in different tissues expression microarray from a set of over 40 k unigenes and gene expression in 19 different tobacco samples has been generated (Edwards et al. 2010). 772 transcription factors previously identified in tobacco were mapped to the array and 87% of them being expressed in at least one tissue in the generated tobacco expression atlas (TobEA). Putative transcriptional

networks were identified based on the co-expression of transcription factors. SGN contains transcriptome sequence collections of *N. sylvestris* (32,276), *N. tomentosiformis* (31,961) and *N. tabacum* (26,284) from transcriptome projects and unigenes data sets of *N. sylvestris* (6,300), *N. tabacum* (84,602) and *N. benthamiana* (16,024).

Large collection of data on nucleotides, genes and protein sequences on *Nicotiana* are available at NCBI site. More than 3 million nucleotide sequences of 20 *Nicotiana* spp. that includes genomic DNA/RNA, mRNA, cRNA, ncRNA, rRNA, tRNA and transcribed RNA are generated by various researchers as on 30.09.2021. Among them, around 895,700 sequences are comprehensive, integrated, non-redundant, well-annotated set of reference sequences including 456,507 ESTs and 1,420,639 genomic survey sequences (GSS). Further, a total of over 201,560 records of gene sequences belonging to 12 *Nicotiana* spp. viz., *N. tabacum, N. tomentosiformis, N. sylvestris, N. attenuata, N. undulate, N. otophora, N. suaveolens, N. glauca, N. stocktonii, N. repanda, N. amplexicaulis and N. debneyi* are available at NCBI website.

Sequence read archive (SRA) data, available through multiple cloud providers and NCBI servers, is the largest publicly available repository of high throughput sequencing data. Nearly, 5,080 records of SRA data of 20 *Nicotiana* spp. are available at NCBI website (as on 30.09.2021). Raw sequencing data and alignment information in SRA are helpful in improving the reproducibility and facilitation of new discoveries through data analysis (https://www.ncbi.nlm.nih.gov/sra). Around 4,860 curated gene expression data sets as well as original series and platform records of 11 *Nicotiana* spp. are available at gene expression omnibus (GEO) repository of NCBI as on 30.09.2021 (https://www.ncbi.nlm.nih.gov/gds). Around 275,000 collection of protein sequences from several sources, including translations from annotated coding regions in GenBank, RefSeq and third party annotation (TPA) Sequence, as well as records from other data bases are available for 20 *Nicotiana* spp. at NCBI.

Genomic resource collection of SGN includes transcriptome sequences, mRNAs, and predicted proteins of five wild *Nicotiana* spp. namely *N. attenuata, N. benthamiana, N. tomentosiformis, N. sylvestris* and *N. otophora* and four versions of *N. tabacum* are available. SGN also hosts 39 transcript libraries of *N. tabacum* and two of *N. sylvestris.* The proteomic data generated globally is stored and can be accessed through the Universal Protein Resource (https://www.uniprot.org/). UniProt database contains 73,606 protein entries associated with *Nicotiana tabacum* proteome (UP000084051) as on 31.03.2021.

10.10.2 Details of Genome Sequencing

10.10.2.1 Nuclear Genome Sequencing

Tobacco has the largest genome size (4.5 Gbp) with large proportion of repetitive sequences compared to other solanaceous crops (tomato, potato, chilli and brinjal) in spite of similar basic chromosome number (n = 12) (Zimmerman and Goldberg 1977;

Arumuganathan and Earle 1991; Kenton et al. 1993; Leitch et al. 2008; Sierro et al. 2014). Even it is 50% larger than that of human genome. In 2003, the first tobacco genome sequencing project was initiated through Tobacco Genome Initiative (TGI) with a purpose of sequencing open reading frames of *N tabacum* cv. Hicks Broadleaf using methyl filtration method of complex reduction (Opperman et al. 2003; Rushton et al. 2008; Sierro et al. 2013b). The sequencing completed in 2007 and sequencing data are available at NCBI Gene Bank (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA29349). However, the genome coverage (689 Mb) was limited because of employment of only enriched genes and portion of genes that are under methylated relative to TEs (Wang and Bennetzen 2015). Later with the advancement of next-generation sequencing (NGS) technologies, sequencing of entire genomes of three cultivated *N. tabacum* as well as 11 wild relatives (*N. knightiana, N. paniculata, N. rustica, N. glauca, N. obtusifolia, N. otophora, N. attenuata, N. sylvestris, N. tomentosiformis, N. undulata* and *N. benthamiana)* have been completed since 2014.

In 2013, the first sequences were released with assemblies of *N. sylvestris* and *N. tomentosiformis* having 94.0 × and 146.0 × genome coverage and a length of 2,222 and 1,688 Mb, respectively (Sierro et al. 2013a). Further, Philip Morris International released three genomes of *N. tabacum,* one at Scaffold level (cv. TN90) and two at contig (cv. K326 and Basma Xanthi-BX) level in 2014 (Seirro et al. 2014). The assembled sequences consist of 3,700 Mb with a GC content of 39%, covering 29-49X using TN 90 as a reference genome.

In 2017, the British American Tobacco released the scaffold level improved version of *N. tabacum* cv. K 326 with a genome coverage of 4,600 Mb of 86X and 33.5% GC content, an increase of 3.6 Gb (i.e. 81% of predicted genome size) compared to previous version (Seirro et al. 2014). Presently, 16 assemblies belonging to 12 *Nicotiana* species (*N. sylvestris, N. tomentosiformis, N. benthamiana, N. tabacum, N. otophora, N. attenuata, N. obtusifolia, N. glauca, N. knightiana, N. paniculata, N. rustica* and *N. undulata*) are available at NCBI genebank with range of 18-146X. Two assemblies of *N. attenuata* and four for *N. tabacum. N. attenuata* reference sequence (2,366 Mb) at chromosome level (12 haploid) while at contig level *N. tabacum* (K326, and Basma Xanthi) and *N. benthamiana* are available. The detail statistics including assembly level and their N50 and L50 values for each genome is also provided at Table 10.2.

Genomic resource collections consisting of five wild *Nicotiana* spp. and four of *N. tabacum* are available at Sol Genome Network (SGN). Under the TGI project, filtered genome sequences generated include contig level using *N. tabacum* cv. BX, *N. tabacum* cv. K326 and *N. tabacum* cv. TN90 as well as improved K326 assemblies of genome scaffolds, proteins and cDNA (Edwards et al. 2017). Further, scaffold level genome assemblies of five *Nicotiana* species (*N. attenuata, N. benthamiana, N. tomentosiformis, N. sylvestris* and *N. otophora*) also information on predicted proteins and mRNA are available at this database.

Table 10.2 Genome sequencing details of *Nicotiana* species as (available at NCBI site)

Genome	Assembly level	Statistics	Genome coverage
N. sylvestris	Scaffold	Total length (Mb): 2,222 GC%: 39.80	94.0x
N. tomentosiformis	Scaffold	Total length (Mb): 1,688 GC%: 39.0999	146.0x
N. benthamiana	Contig	Total length (Mb): 62 GC%: 39.6	79x
N. tabacum cv. TN90 (RefSeq Genome)	Scaffold	Total length (Mb): 3,643 GC%: 39.2006	49.0x
N. tabacum cv. K326 (sequencing and assembly)	Contig	Total length (Mb): 3,733 GC%: 39	38.0x
N. tabacum cv.: BX (sequencing and assembly)	Contig	Total length (Mb): 3,736 GC%: 39	29.0x
N. otophora	Contig	Total length (Mb): 2,689 GC%: 39.1	66.0x
N. attenuata strain: UT (RefSeq, sequencing and assembly)	Chromosome (12 haploid)	Total length (Mb): 2,366 GC%: 41.333	30.0x
N. obtusifolia	Scaffold	Total length (Mb): 1,223 GC%: 38.4	50.0x
N. attenuata ecotype Arizona (sequencing and assembly)	Scaffold	Total length (Mb): 1,828 GC%: 29.1	18.0x
N. tabacum cv. K326 (sequencing and assembly)	Scaffold	Total length (Mb): 4,647 GC%: 33.5	86x
N. glauca	Scaffold	Total length (Mb): 3,223 GC%: 36.6	35.0x
N. knightiana	Scaffold	Total length (Mb): 2,299 GC%: 39.7	82.0x
N. paniculata	Scaffold	Total length (Mb): 2,191 GC%: 39.4	100.0x
N. rustica	Scaffold	Total length (Mb): 4,231 GC%: 40	90.0x

(continued)

Table 10.2 (continued)

Genome	Assembly level	Statistics	Genome coverage
N. undulata	Scaffold	Total length (Mb): 1,914 GC%: 40	112.0x

10.10.2.2 Organelle Genome Sequencing

Chloroplast and mitochondrial genomes of tobacco are circular DNA molecules. The size of the plastid genome of *Nicotiana* species is 0.156 Mb. Shinozaki et al. (1986) for the first time sequenced the chloroplast genome of tobacco in 1986. Presently, sequencing of about 219 plastid genomes have been completed including 12 Popset data (DNA sequences derived from population, phylogenetic, mutation and ecosystem studies) related to five *Nicotiana* species. In addition, recently, Mehmood et al. (2020) assembled the plastid genomes and compared the five tobacco species namely *N. knightiana* (155,968 bp), *N. rustica* (155,849 bp), *N. paniculata* (155,689 bp), *N. obtusifolia* (156,022 bp) and *N. glauca* (155,917 bp). Reference plastid genomes of five *Nicotiana* species namely *N. tabacum* (155,943 bp), *N. attenuata* (155,886 bp), *N. tomentosiformis* (155,745 bp), *N. sylvestris* (155,941 bp), *and N. otophora* (156,073 bp) are available at NCBI (Table 10.2).

Starting from 2003, till date sequencing of eight mitochondrial complete genomes consist of five popsets of three *Nicotiana* species namely *N. tabacum* (430,597 bp), *N. attenuata* (394,341 bp) and *N. sylvestris* (430,597 bp) are completed and the details are available at NCBI site (Table 10.3). Further, reference mitochondrial genomes are made available for *N. tabacum, N. attenuata* and *N. sylvestris* at the NCBI.

Table 10.3 *Nicotiana* organelle genome details available at NCBI (as on 31.03.2021)

Organelle reference genome	PopSet	Archived genomes	Sequence length (bp)	Genes	Proteins
Plastid					
N. tabacum *(RefSeq)*	10	124	155,943	144	98
N. attenuata	1	15	155,886	129	84
N. tomentosiformis	–	30	155,745	150	102
N. sylvestris	1	42	155,941	149	101
N. otophora	–	8	156,073	155	108
Mitochondrial					
N. tabacum	4	6	430,597	183	153
N. attenuata		1	394,341	68	40
N. sylvestris	1	1	430,597	64	37

10.10.3 Gene Annotation

Genome annotation aims at identifying functional elements along the sequence of a genome. Once a genome is sequenced, it must be annotated to understand its functions for its further successful use in genetic manipulation. In tobacco, both nuclear and organelle genomes are successfully annotated. Gene annotation records of *Nicotiana* sp data available at NCBI and SGN databases are presented in Tables 10.4 and 10.5.

N. sylvestris (2014) was the first species annotated followed by *N. tabacum* cv. TN 90 (2016), *N. attenuata* strain UT (2016) and *N. tomentosiformis* (2020). Presently, annotation reports of four *Nicotiana* species viz., *N. sylvestris*, *N. tabacum* cv. TN 90, *N. attenuata* strain UT and *N. tomentosiformis* are available at NCBI website (Table 10.4).

Predictions of genes annotated are made available for published genomes of *N. attenuata*, *N. benthamiana, N. tomentosiformis, N. sylvestris* and four versions of *N. tabacum* at SGN site also (Table 10.5). For *N. tabacum,* predicted proteins were ranging from 69,500 to 122,388 and mRNA from 145,503 to 189,413 in *N tabacum.*

Table 10.4 Gene annotation reports of *Nicotiana* species (as per NCBI)

Species	*N. tabacum*	*N. sylvestris*	*N. tomentosiformis*	*N. attenuata*
Genome assembly name	Ntab-TN90	Nsyl	Ntom_v01	NIATTr2
Annotation release number	100	100	102	100
Release date	04-05-2016	22-10-2014	21-04-2020	06-12-2016
RefSeq scaffolds	168,245	253,917	159,547	37,135
mRNAs	84,001	48,059	50,010	44,491
Genes and pseudogenes	73,946	40,317	45,485	39,977
– protein-coding	61,526	33,678	31,842	34,094
– non-coding	9,019	4,667	11,549	3,886
– pseudogenes	3,401	1,972	2094	1,997
– genes with variants	14,549	9,033	10,545	6,946

Table 10.5 Gene annotation details of tobacco species (as per SGN)

Nicotiana species	Reference scaffolds	mRNA	Predicted proteins
N. tabacum cv. K326	382,373	145,503	85,994
N. tabacum cv. BX	420,216	146,748	86,009
N. tabacum cv. TN90	249,104	189,413	122,388
N. tabacum v4.5 (Edwards et al. 2017)	1,084,432	–	69,500
N. tomentosiformis	159,548	85,853	53,753
N. sylvestris	253,917	87,234	54,497
N. benthamiana	56,094	–	57,140
N. attenuata	37,182	33,449	33,449

However, a smaller number of proteins (33,449–54,497) and mRNA (33,449–87,234) were predicted for *Nicotiana* species compared to *N. tabacum.*

Uniprot database of *N. tabacum* (UP000084051) contains 73,606 protein entries (https://www.uniprot.org/) as on 31.03.2021. Edwards et al. (2017) identified predicted proteins exhibiting cross-over with the related Solanaceae species like tomato and potato in addition to other flowering plants based on gene ontology analysis.

Annotations are also available for published organelle genome sequences (Table 10.3). Predicted plastid genes in five *Nicotiana* spp. vary from 129 to 155 and proteins from 84 to 108. The predicted genes and proteins for mitochondrial genome are more for cultivated species, *N. tabacum* (183; 153, respectively) compared to wild species, *N. attenuata* (68; 40) and *N. sylvestris* (64; 37).

10.10.4 Impact on Germplasm Characterization and Gene Discovery

Availability of sequencing information of *Nicotiana* Species made it possible to compare sequences within *Nicotiana* Species and also with other solanaceous crops. Thus, the comparative genomics helped to understand the relationships between cultivated and wild species and their progenitors in terms of their sequence similarity and genome rearrangements (Wu et al. 2009; Sierro et al. 2014; Gong et al. 2016; Asaf et al. 2016; Edwards et al. 2017). Homologous genes were identified between the genomes of *N. tabacum* cv. TN90, K326 and BX and other solanaceous crops like tomato and potato (Sierro et al. 2014).

Gene annotation using published genome sequences has helped in identification of functional sequences and predicted mRNAs and proteins that can be expressed in tobacco. Sierro et al. (2014) recognized the genome assemblies and genomic regions responsible for virus resistance in draft genomes. The *N glutinosa N* gene, source of TMV resistance in tobacco was found in the draft genome sequence of TN 90 cultivar and weak identity in susceptible genome of K326 and BX genome. Thus, genes responsible for abiotic stresses can also be identified using genome sequence information.

Using the available data on sequences of *Nicotiana* genomes and EST, large number of SSRs and SNPs were identified (Bindler et al. 2011; Tong et al. 2012, 2020; Cai et al. 2015; Xiao et al. 2015; Thimmegowda et al. 2018; Wang et al. 2018). The potential markers have been extensively used for characterization of germplasm and DUS testing for assessment of genetic diversity and genetic relatedness among cultivated varieties (Moon et al. 2008, 2009a, b; Davalieva et al. 2010; Fricano et al. 2012; Gholizadeh et al. 2012; Prabhakararao et al. 2012; Xiang et al. 2017; Binbin et al. 2020).

Core markers developed based on genotyping-by-sequencing were used for varietal identification and fingerprinting of cigar tobacco accessions (Wang et al. 2021).

The high-density maps developed based on SSR and SNP markers will be useful for germplasm characterization and identification of target traits including abiotic stresses.

Genome-wide SNP markers were identified and used for association analysis of leaf chemistry traits in natural populations of tobacco germplasms (Tong et al. 2020). The high density genetic maps developed in tobacco facilitate the tobacco genetic researchers to detect genome-wide DNA polymorphisms, fine map and clone their trait of interest. Genome-wide DNA polymorphisms could also be identified using the custom 30 K Infinium iSelect HD Bead Chip SNP chip (Edwards et al. 2017). Map based cloning of two homeologous candidate genes conferring white stem phenotype in recessive condition in burley tobacco proved that map based cloning of target traits is possible in tobacco (Edwards et al. 2017).

Discovery of novel genes/alleles for any given trait could be achievable with genotyping-by-sequencing and whole-genome re-sequencing methods. Genomics tools can be used for rapid identification and selection of novel beneficial genes and their incorporation into cultivated species.

Genome-wide association studies (GWAS) could be used to identify the novel genomic regions governing traits of interest by associating between DNA polymorphisms and trait variations in diverse germplasm collections that are phenotyped and genotyped.

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

The latest advances in high throughput sequencing technologies and accurate phenotyping platforms are helpful in transforming molecular breeding to genomic assisted breeding (GAB). GAB has key role in developing future tobacco cultivars with rapid accumulation of beneficial alleles and removing deleterious alleles (Varshney et al. 2021). With the availability of *Nicotiana* draft genomes, transcriptome and metabolome profiles might be helpful in understanding the genomic locations, ancestral relationships, gene products and expression patterns responsible for abiotic stresses. Development of genetic maps (Sect. 10.7) using available molecular markers (Sects. 10.5.2 and 10.7.2) and identification of trait specific linked DNA markers with key abiotic stress tolerant traits can pave the way for the utilization of GAB in tobacco improvement.

Presently, in tobacco application of structural and functional genomics in GAB and application of DNA markers for MAS is in infant stage. Considerable progress made in genomics and generation of genotypic and phenotypic populations can be used to develop predictive models for estimation of breeding values for genomic selection. Breeding value can be used to predict the performance of parents in crossing and genetic advance in breeding lines based on genomic profile of target population in the target environment. Early generation selection of desirable lines without much

effect of environment can shorten the time required for breeding. The genotypic data generated from seed or seedling and the favorable alleles can be used to predict the performance of mature individuals without extensive phenotyping over years in different environments during GAB (Varshney et al. 2014).

10.11 Recent Concepts and Strategies Developed

Gene editing and nanotechnology are emerging as important tools for genomic designing in crop plants for various traits including abiotic stress tolerance.

10.11.1 Gene Editing

Gene editing involves precise modifications in the target genes to bring desirable changes in the phenotypes of organism. Gene editing technology alter gene expression of gene of interest through structural and epigenetic modifications of the DNA of target gene through various techniques like nuclease technologies, homing endonucleases, and certain chemical methods (Khan 2019). Other molecular techniques like meganuclease (MegaN), transcription activator-like effector nucleases (TALENs), and zinc-finger nucleases (ZFNs) have also emerged as important gene-editing technologies (Townsend et al. 2009). These initial technologies have limitation of lower specificity due to their off-targets side effects. The latest discovery of the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) nuclease system seems more efficient in improving the efficiency and feasibility to taking the genome-engineering techniques to the next higher level of molecular engineering (Ahmer et al. 2021). Either *Agrobacterium*-mediated or protoplast transformation are routinely used delivery systems in plants in most of the gene editing technologies. Editing of target genes was successfully demonstrated in tobacco using meganucleases (Puchta 1999; Honig et al. 2015), ZFNs (Townsend et al. 2009), TALENs (Zhang et al. 2013) and CRISPR/Cas9 system (Hirohata et al. 2019; Tian et al. 2020, 2021). These promising techniques can effectively be used in the improvement of tobacco for abiotic stress tolerance in future.

10.11.2 Nanotechnology

The genetic engineering technique basically modifies plant cell genomes, through efficient delivery of modifier biomolecules such as DNA, SiRNA and miRNA as genetic cargo to targeted plant (Cunningham et al. 2018; Ahmar et al. 2021). *Agrobacterium*-mediated transformation (AMT), biolistic delivery of DNA, electroporation, viral vectors and chemical delivery are most widely used delivery systems. Random

DNA integration, disruption of endogenous plant genes, variable gene expression arising out of inserted sites, tissue damage, cytotoxicity, requirement of protoplasts regeneration, less cargo sizes, high host specificity etc. are one or the other limitations associated with these systems (Niazian et al. 2017; Toda et al. 2019). These limitations can be effectively overcome by advanced nanotechnological tools.

Nanoparticles (NPs) are more useful for delivery of various genetic cargo (DNA, RNA, proteins and ribonucleoproteins across plant systems in species independent passive manner (Cunningham et al. 2018). Thus, NP mediated delivery systems successfully used for increasing the efficiency, robustness and versatility of genetic engineering through use of nanocarriers by forming a binding complex with biomodifier molecules (CRISPR/Cas) while delivering into plant cells (Demirer et al. 2021). This incomparable potential of the NP based delivery of biomolecules to plant cells has revolutionized the gene delivery systems (Ahmer et al. 2021) and emerged as cutting-edge technology that provides new insights and robustness of gene editing technology (Cunningham et al. 2018). Various barriers like species- and tissue-specific limitations of delivery of biomolecules to plant cells can be overcome by NPs because of smaller size and transverse the cell wall. Further NPs can also be engineered to mediate cargo delivery to various subcellar parts, where even AMT cannot target such as mitochondrial or chloroplast DNA.

NPs system of biomolecular delivery in both plant and animals are classified into five types based on the base material use namely bio-inspired, carbon-based, silicon-based, polymeric, and metallic/magnetic (Cunningham et al. 2018). Each NP type delvers different genetic cargos viz., carbon nanotubes (CNTs) can carry RNA and DNA; metallic NPs can only deliver DNA as genetic cargo; silicon-based NPs can carry DNA and proteins; polymeric NPs (e.g., PEG and polyethyleneimine) can transfer encapsulated RNA, DNA, and proteins into cells (Silva et al. 2010).

Cationic NPs type can bind to the plant cell wall (negatively charged) and successfully be used for gene transfer, whereas CNT NPs have been used to deliver plasmid DNA into various crops.NP mediated cargo delivery can be done either in physical or non-physical means. Physical methods include creating transient pores in the cell membrane with electric fields, soundwaves, or light, magnefection, microinjection, and biolistic particle delivery. Non-physical methods embrace the use of cationic carriers, incubation, and infiltration. Various NPs can behave differently in specific plant cells, which require optimizing their application for different plant species and their dose and spatiotemporal tuning (Ahmar et al. 2021). The use of NPs for plant transformation enables an efficient method because NPs protect the genetic cargo from cellular enzymatic degradation (Ahmar et al. 2021).

Literature on NP mediated transformation system in tobacco mainly focusing on genetically modified tobacco mosaic virus-based metallic nanomaterial synthesis, NPs as pesticides, NP uptake, effects on plant growth, biomolecule delivery systems etc. (Burklew et al. 2012; Wang et al. 2016; Tirani et al. 2019). Torney et al. (2007) was first to demonstrate NP co delivery of DNA and chemicals in *N tabacum* plants via biolistic delivery of 100–200 nm gold capped mesoporous silica NPs (MSNs).

Zinc NPs were used to deliver DNA plasmid into tobacco (Fu et al. 2012). Silicon carbide whiskers (SCW) and MSN, have been effectively used to transfer genes

into tobacco without using other physical methods (Golestanipour et al. 2018). In general, the SCW method has one disadvantage compared with other NP-mediated plant transformation in that an adequate protocol is required for plant regeneration from cell cultures. Silva et al. (2010) used polymer NPs to introduce siRNA into tobacco protoplasts, providing an alternative gene knockout mechanism in plant cells. Meanwhile, NP-mediated passive delivery of DNA plasmids has been reported with tobacco through CNTs (Burlaka et al. 2015; Kwak et al. 2019) and dsDNA through clay nano sheet NPs (Mitter et al. 2017). Demirer and colleagues (2019) have recently achieved passive delivery of DNA plasmids and protected siRNA using functionalized CNT NPs for transient silencing of constitutively expressed gene in transgenic *N. benthamiana* leaves with 95% efficiency.

With significant advantages, use of NPs in GE, possess few challenges like nanophytotoxicity effect on plant growth and environment (Ahmar et al. 2021). Cell structural stability and metabolic pathway disturbance and deposition and dispersal to other plant cells after application needs further research. Another limitation is efficient binding of biomolecules to NPs and the disintegration of the binding complex in plant cells because of different binding affinities with NPs based on their structure, charge, chemical composition, and surface area, making them ideal for a bioconjugation complex. So, their optimization for binding specific biomolecules requires further research to enhance their versatility as genetic cargo.

10.12 Brief on Genetic Engineering for Resistance Traits

Tobacco is extensively used as a model plant system in genetic engineering research (Jube and Borthakur 2007) for the study of basic biological functions, such as plant-pathogen interactions, environmental responses, growth regulation, senescence, etc. In view of this, number of studies have been undertaken incorporating genes from bacteria, animals and other plant species into tobacco and their functional roles validated. Genetic engineering of tobacco plants for resistance related traits are reviewed here.

10.12.1 Target Traits and Alien Genes

Tobacco was used as a model system for the functional validation of a number of abiotic stress responsive genes from different crop plants through their transgenic incorporation into tobacco. Large volume of information is available in literature on this area. Target traits for imparting resistance to various abiotic stresses that are validated in tobacco are briefly detailed here under.

10.12.1.1 Drought Tolerance

A number of drought resistant candidate gene were transferred to tobacco to study improved drought tolerance (Kolodyazhnaya et al. 2009). These include DREB and WRKY transcription factors, genes that alter the levels of trehalose and mannitol, and LEA genes (Tarczynski et al. 1992, 1993; Pilon-Smits et al. 1998; Kasuga et al. 2004; Wang et al. 2006; Wei et al. 2008; Rabara et al. 2015). Further, a number of reports on tobacco transgenics incorporated with novel genes/gene products/transcription factors viz., RING-finger protein (RFP1), NAC transcription factors 2a, atriplex hortensis choline monoxygenase (AhCMO), Δ1-pyrroline-5-carboxylate synthetase (P5CSF129A), neomycine phosphotransferase (nptII), pyrroline-5-carboxylate synthetase (P5CS), NADP-ME, Cox, Mannitol-1-phosphate dehydrogenase (mt1D), pleurotus sajor-caju trehalose, phosphorylase (PsTP), Trehalose-6-phosphate synthase (TPS1), *Triticum aestivum* ubiquitin 2, Boea hygrometrica late embryogenesis and abundant proteins (BhLEA1 BhLEA2) for validating their role in incorporating drought resistance are available in literature.

10.12.1.2 Salinity Tolerance

Salt stress tolerance responses induces extensive gene expression of various genes viz., ion transport, antioxidant systems, hormonal regulators and autotransporters. The overexpression of various genes from various sources showed tolerance against salinity stress in tobacco. Gene/gene products viz., Na+/H+ antiporter (SOS1) and *PPase* TVP1 for altered accumulation of Na+ and K+ in shoot and root; CDH, BDH, COX, PEAMT genes for osmo- protection through glycine betaine proved enhance salt tolerance. During the evolution halophytes developed various morphological, anatomical, and physiological mechanisms to sustain under salinity condition. The salt responsive genes/antiporters (SeCMO, SbGSTU, SbNHX1, AINHX, AISAP) from halophytes (*Salicornia* spp. and *Aleuropus* spp.) have been well characterized and extensively used to develop salt stress tolerant transgenic tobacco.

10.12.1.3 Heat Tolerance

Thermo tolerance is a complex multigenic trait, which is influenced by genotype × environment interactions. Heat shock proteins (Hsps) are molecular chaperones that maintains vast range of cellular functions ranging from protein aggregation and folding of proteins to membrane stability and transcription factors under extreme temperature conditions. Transgenics possessing heat tolerance have been developed using different genetic engineering techniques in tobacco. Various members of *HSPs* (*HsP* 101, *HsP* 70, *HsP* 16.9 and *HsP* 18.2) and several other genes/plant proteins such as FAD 7, Dank 1, ubiquitin, cytosolic Cu/Zn-SOD and Mn-POD have been characterized and developed transgenic plants showing enhanced tolerance to heat/cold stress in tobacco. Over expression of cytokinin oxidase/dehydrogenase

(CKX1) gene, modification of fatty acids in thylakoid membranes of tobacco chloroplasts osmoprotectants in transgenic tobacco found to impart temperature tolerance in tobacco.

10.12.1.4 Cold Tolerance

Considerable number of cold responsive genes and gene regulated networks have been reported in tobacco. Several studies have showed that transgenics developed with transformation of novel cold responsive genes from different sources (*Casell* spp., *Brachypodium* spp., *Populus* spp.) imparts cold tolerance through increase in the activities of leaf malondialdehyde, superoxide dismutase and antioxidant enzyme activity, and increased accumulation of inositol etc. in tobacco.

10.12.1.5 Water Logging Tolerance

Water logging condition triggers a series of morpho biochemical changes and gene expression leading to adaptation to the stress. Various regulatory networks and transcription factors were involved to adapt to water logging and low oxygen. The transgenes from Brassica *oleracea* and *Actinidia deliciosa* proved to accumulate more dry matter, leaf chlorophyll content and fresh and dry weight in tobacco under waterlogging conditions.

10.12.1.6 Heavy Metal Tolerance

To cope with heavy metal tolerance, plants employ various strategies which involve complex physiological and biochemical changes including changes in global gene expression to cope up with heavy metal tolerance. Recently nickel, cadmium and aluminum toxicity were addressed in tobacco through genetic engineering approaches using novel stress responsive gene like AtDHAR and SbMYB15 transcription factors.

10.12.1.7 Engineering Multiple Stress Tolerance in Tobacco

Stress tolerance mechanism in plants is controlled by complex transcriptional network and transcription factors (TFs) are the major player in this network. The cascade of molecular responses consists of perception of stress, transduction of signals to cellular machinery, gene expression, metabolic changes lead to stress tolerance. Plants exhibit both, stress-specific and correlated other shared responses that may protect them from several environmental stresses. Recent developments of stress biology showed evidence of crosstalk between abiotic and biotic stress responses in biological systems. Novel functional genes possessing multiple stress resistance have

been transformed into the tobacco plants successfully and their phenotypic effect were determined.

10.12.2 Review on Achievements of Transgenics

Tobacco has served as a model plant for producing large number of transgenics having abiotic stress tolerance and other economically important genes. However, no genetically transformed tobacco varieties (transgenic cultivars) are released for commercial cultivation in any of the countries, in view of the opposition faced by genetically modified (GM) tobacco in the global market (Bowman and Sisson 2000). Though GM Approval Database of International Service for the Acquisition of Agri-biotech Applications (ISAAA) reports two GM tobacco events viz. (1) oxynil herbicide tolerance and (2) nicotine reduction, antibiotic resistance (GM approval database 2021), none of them are cultivated on commercial scale in any of the countries. In contrast, millions of hectares of genetically engineered soybean, corn, cotton and canola are being grown throughout the world (ISAAA 2019). Thus, tobacco breeding efforts lag behind those of other crops in genetic engineering. In addition, the strong opposition from the European countries to genetically modified organisms (GMOs) is also acting as hindrance in transgenic tobacco breeding. Thus, genetic engineering of tobacco cultivars is on hold until the trade related obstacles are alleviated. However, this methodology holds great promise for improving tobacco cultivars in terms of disease and pest resistance, and possibly health-related constituents in the cured leaf.

10.12.3 Organelle Transformation

As organelles, plastids and mitochondria, containing genetic materials in small DNA genomes provide an opportunity for transformation in plants (Butow and Fox 1990). Plastid genomes of tobacco are typically 150 kb, and codes for about 140 genes. Plastids are the site of most important biosynthetic processes and pathways, such as photosynthesis, photorespiration, metabolism of amino acids, lipids, starch, carotenoids, other isoprenoids, phenol compounds, purines, pyrimidines, isoprenoids, starch, pigments, vitamins synthesis, and also are implicated in the metabolism of phytohormones such as cytokinins, abscisic acid, and gibberellins (Kuchuk et al. 2006; Rascon-Cruz et al. 2021). Compared with conventional nuclear genetic engineering, plastid genome transformation offers several benefits (Kuchuk et al. 2006; Li et al. 2021). High level of transgene expression is possible with chloroplasts as there are about 100 chloroplasts per cell, each containing about 100 copies of genome. Thus, there is possibility of 10,000 copies of transgenes per cell due to plastid transformation. Gene silencing, or the so-called position effects observed in nuclear transformation were not described for plastid genes. Hence, the level of expression is much

more predictable. Unlike integration into the nuclear genome, integration of heterologous DNA into a plastome occurs via a homologous recombination mechanism, thus allowing very precise genetic manipulations. Multigene engineering through stacking transgenes in synthetic operons in a single transformation event is possible through plastid transformation. Maternal inheritance of plastomes avoid the risk of uncontrolled transgene release into the environment (Kuchuk et al. 2006; Li et al. 2021).

Stable transformation of the plastome was first achieved in tobacco by Svab et al. (1990). Over the years, plastid transformation in tobacco has become more and more routine, with a transformation efficiency approaching that of nuclear transformation (Svab and Maliga 1993; Daniell et al. 2016; Li et al. 2021). Plastids of *N. tabacum* var. Petit Havana (Svab et al. 1990), *N. benthamiana* (Davarpanah et al. 2009) and *N. sylvestris* (Maliga and Svab 2010) were successfully transformed by different workers. The development of plastid transformation technology has paved the way to transgene expression, genome editing, and RNA editing analysis in plastids.

Though possibility of transformation exists for mitochondrial genome, reliable methods for the transformation of mitochondria using a biolistic device currently exist only for yeasts (Johnston et al. 1988) and green algae (Remacle et al. 2006). No successful transformation of mitochondria in plant systems has been reported to date (Li et al. 2021). A genetic transformation system for plant mitochondria would allow functional analyses of the mitochondrial genome and its products, and would open the way for engineering of the genome to modify mitochondrial metabolism, or to introduce cytoplasmic male sterility (CMS) into new crops and varieties (European Commission 1989; Wang et al. 2020).

10.12.4 Biosynthesis

Major changes occur in physiology, metabolites, mRNA levels, and promoter activities during the tobacco response to abiotic stresses (Rabara et al. 2015). Components of a core metabolic response at the gene, metabolite, plant hormone, transcription factor, and promoter levels and are regulated by family-specific changes in transcription factor activity. Numerous biochemical pathways leading to tens of thousands of primary and secondary metabolites are involved in abiotic stress resistance in plants (Nascimento and Fett-Neto 2010). Primary metabolic pathways producing sugars (trehalose, sucrose and fructan), amino acids (tryptophan and proline), and ammonium compounds (polyamines and glycine betaine) serve as osmoprotectants under stressed condition. Enhanced biosynthesis of these osmoprotectants will improve the abiotic stress tolerance (Rathinasabapathi 2000; Rontein et al. 2002).

10.12.5 Metabolic Engineering Pathways and Gene Discovery

A metabolic pathway is defined as any sequence of feasible and observable biochemical-reaction steps connecting a specified set of input and output metabolites. The pathway flux is the rate at which input metabolites are processed to form output metabolites. Metabolic engineering involves beneficial alteration of metabolic pathways using recombinant DNA technology to better understand and utilize the cellular pathways for the production of useful metabolites (Bailey 1991). This method involves overexpression or downregulation of certain proteins in a metabolic pathway, such that the cell produces a new product.

Complete understanding of metabolic pathway and genes involved in the path way and host cell for genetic modifications are essential for the successful engineering (Fuentes et al. 2018). Multiple transgenes expressing more than one gene involved in the pathway are frequently required in metabolic engineering which is a challenge with traditional transgenic approaches. New technological options such as combinatorial transformation (large-scale co-transformation of the nuclear genome) and transformation of the chloroplast genome with synthetic operon constructs (Bock 2013) offers straight forward multigene engineering by pathway expression from operons, high transgene expression levels, and increased transgene containment due to maternal inheritance of plastids. It also provides direct access to the large and diverse metabolite pools available in chloroplasts and non-green plastid types.

The transcription factors are often present as gene families and regulate target genes in tissue- and species-related patterns (Bovy et al. 2002). Transcription factors (TFs) tend to control multiple pathway steps and hence, considered as powerful tools for the manipulation of complex metabolic pathways for engineering the levels of metabolites (Broun 2004; Grotewold 2008). Analysis of changes in transcriptomes and metabolomes should provide clues related to regulation by transcription factors in heterologous systems. As a group, flavonoids are involved in many aspects of plant growth and development, such as pathogen resistance, pigment production, UV light protection, pollen growth, and seed coat development (Harborne 1986). Hence, manipulation of phenylpropanoid pathway responsible for flavonoid production can be a strategy for biotic and abiotic stress resistance.

Metabolic engineering was successfully demonstrated for enhancing the target molecules in tobacco altering multiple genes through plastid transformation (Lücker et al. 2004; Lu et al. 2013, 2017). Grafting the transplastomic tobacco onto the non-transformable species *N. glauca* facilitated the horizontal transfer of the transgenic plastid genomes across the graft junction (Lu et al. 2017). Thus, grafting may be helpful in the transplastomic engineering of plant species that are otherwise not amenable. Metabolic engineering of artemisinic acid biosynthetic pathway provided a proof of concept for combining plastid and nuclear transformation to optimize product yields from complex biochemical pathways in chloroplasts (Fuentes et al. 2016). Transplastomic tobacco that expressed the core artemisinic acid biosynthetic pathway from two synthetic operons accumulated only low levels of the metabolite.

However, super transformation of the transplastomics lines using the COSTREL (combinatorial super transformation of transplastomic recipient lines) approach, increased the artemisinic acid content up to 77-fold. Reduction in photorespiration could be obtained through the introduction of three distinct alternative glycolate metabolic pathways into tobacco chloroplasts (South et al. 2019). Coupling the alternative photorespiratory pathway with reduced expression of a glycolate and glycerate transporter to limit glycolate flux out of the chloroplast raised biomass productivity by >40% under field conditions (South et al. 2019). In this study, a total of 17 constructs were designed for nuclear transformation; these multienzyme pathways could potentially be introduced into the chloroplast by plastid transformation in the form of operons.

10.12.6 Gene Stacking

Gene stacking or gene pyramiding or multigene transfer refers to incorporation of two or more genes of interest into a single plant. The combined traits resulting from this process are called stacked traits. A biotech crop variety that bears stacked traits is called a biotech stack or simply stack (ISAAA 2020). Biotech stacks are engineered to overcoming the myriad of problems such as insect pests, diseases, weeds, and environmental stresses so that farmers can increase their productivity. Insect resistance based on multiple genes confers stable resistance than single gene which may breakdown due to co-evaluation of pests. The main methods for genetically engineering plants with gene stacking involve (i) the simultaneous introduction, by the co-transformation process, and (ii) the sequential introduction of genes using the re-transformation processes or the sexual crossing between separate transgenic events.

Though, stacked products are promising and technically feasible in tobacco (Bakhsh et al. 2018; Li et al. 2018; Boccardo et al. 2019), till date, none of the stacks are approved for commercial cultivation in tobacco mainly because of their transgenic tag. Gene pyramiding events in tobacco are mainly used as proof of concepts or for gene function and interaction studies. Regulatory principles and procedures for approval and release of biotech stacks differ globally (ISAAA 2020). No separate or additional regulatory approval is necessary in countries like the USA and Canada for commercializing hybrid stacks developed through crossing a number of already approved biotech lines. This policy is based on the argument that interactions between individual trait components in a stack that have been shown to pose no environmental or health hazard would not result in new or altered hazards. However, in Japan and European Union the stacks are considered new events, even if individual events have market approval, and must pass through regulatory approval process including safety assessment (ISAAA 2020). Risk assessment of stacks is focused on the identification of additional risks that may arise from the combined genes.

10.12.7 Gene Silencing

Gene silencing is the prevention or reduction in the expression of a certain gene through regulation of its gene expression in a cell. Gene silencing strategies are particularly useful in functional characterization of abiotic stress resistant genes. RNA interference (RNAi)-based silencing of stress responsive genes and studying the knockdown plants for their response to stress can be options for assessing functional significance of these genes and their utilization (Senthil-Kumar et al. 2010). Functional characterization of stress responsive genes helps to understand the role of specific genes in stress tolerance so as to manipulate them in designing stress resistant cultivars. Primarily, the mechanism of RNAi mediated gene silencing is based on the exogenous production of short interfering RNAs/microRNAs (siRNAs/miRNAs) by an organism to control the expression of genes. Expression or introduction of double-stranded (ds) RNA in eukaryotic cells can trigger sequence-specific gene silencing of transgenes and endogenes.

10.12.8 Prospects of Cisgenics

In cisgenesis, the extra DNA originates from a donor plant with which the acceptor plant can cross-breed (Schouten et al. 2006). Combining both traditional breeding techniques and modern biotechnology, this approach dramatically speed up the breeding process. Introduction of desired genes through cisgenesis overcome the linkage drag and prevents hazards such as induced translocation or mutation breeding (Telem et al. 2013; Hou et al. 2014). Using cisgenesis both abiotic and biotic stress resistance genes can be pyramided to provide broader and long-lasting forms of resistance. Cisgenesis reduces the time required for transferring a single gene or more so with multiple genes compared to conventional breeding that require several backcrossed generations to remove undesired genes (Telem et al. 2013).

Introduction of exogenous transfer process related genes in cisgenesis can be avoided with the use of new transformation protocols without bacterial selection markers and use of species-specific plastic DNAs (P-DNAs) instead of bacterial T-DNAs for insertion of isolated genes (de Vetten et al. 2003; Rommens et al. 2004; Schaart et al. 2004). Techniques such as promoter trapping and RNA fingerprinting for the isolation of native regulatory elements can be exploited for the precise expression of target traits (Meissner et al. 2000; Trindade et al. 2003). Majority of the methods for production of cisgenic crops have been patented, and therefore scientists need appropriate approvals to use these patents or design new methods to eliminate the undesired DNA sequences from host genomes (Holme et al. 2013).

The prospects for cisgenesis are enormous in tobacco crop in view of the availability of large number of wild species and germplasm resources, genome sequence information of cultivated tobacco and few wild relatives, and comparative genomic techniques, the development of efficient gene isolation techniques like map-based

cloning and allele mining for identification and cloning of abiotic stress resistant traits from tobacco and their wild relatives. Cisgenesis was successfully demonstrated in tobacco through various gene editing techniques such as ZFNs (Townsend et al. 2009), TALENs (Zhang et al. 2013) and CRISPR-Cas (Upadhyay et al. 2013; Ali et al. 2015). Single or multiple biotic stress resistant cisgenes can be successfully identified, cloned and transferred into tobacco in future with the ever-improving gene technologies.

Cisgenesis introduce only genes of interest from the plant itself or from a crossable species which otherwise could also be transferred by traditional breeding techniques. Hence, cisgenesis is more similar to traditional plant breeding than transgenesis. Release of cisgenic plants into the environment is as safe as that of traditionally bred plants and there is no environmental risk evoked. Therefore, cisgenic plants can be considered as non-transgenic, in spite of using the methods of genetic engineering. There is a need to distinguish cisgenesis from transgenesis as any restrictions on cisgenesis could hamper further research and application of improved crop varieties, especially at a time when more number of genes from crops and their crossable wild relatives are being isolated.

Surveys indicated that cisgenic plants are more acceptable to common people than transgenic plants (Viswanath and Strauss 2010; Gaskell et al. 2011; Mielby 2011). However, GMO regulations in majority of the countries do not distinguish transgenic plants from cisgenic plants. Product-based regulation system rather than a process-based one followed in Canada making it legally possible to control cisgenic plants less strictly compared to transgenic plants. In Australia, cisgenic plants are treated differently under GMO regulations (Russell and Sparrow 2008). While, European Food Safety Authority (EFSA 2012) validated that cisgenic plants are similar to the traditionally bred plants in terms of environment, food and feed security.

In spite of the availability of abiotic stress resistant tobacco trasgenics, worldwide GMO regulations making it difficult to utilize them for commercial cultivation. In such situation, differential treatment to cisgenics are treated differently, that will boost the cisgenesis research in tobacco for improving tobacco yields and resistant factors.

10.13 Brief Account on Role of Bioinformatics as a Tool

Advances in sequencing technology applications have resulted in the accumulation of large volumes of biological data in terms of nucleic acid sequences. To store and analyze these data, number of general and crop specific databases were created. The databases may contain the information covering one or more than one type of omics in an integrated way. The information pertaining to tobacco are being stored and accessed through quite several databases, globally. However, some of the important key databases that are covering tobacco data information are discussed here under.

10.13.1 Gene and Genome Databases

Advances in sequencing technologies, gene mapping and tagging projects, and phylogenetic studies have resulted in accumulation of large volumes of genomic data in tobacco. The genomic databases serve as hubs for storing, sharing and comparison of accumulated data across research studies, data types, individuals and organisms.

Among the various databases, the key genome databases harboring *Nicotiana* genome and gene information are NCBI Genome, Sol Genome Networks (SGN), Kyoto Encyclopedia of Genes and Genomes (KEGG genome), EnsemblPlants, *Nicotiana attenuata* data hub (NaDH), The International Nucleotide Sequence Database Collaboration (INSDC), Gramene etc. (Table 10.6). NCBI and SGN together are the important databases that covers all the information on genomes and genes of various *Nicotiana* species. At present, genome sequences of 12 *Nicotiana* spp. viz., *N. tabacum, N. tomentosiformis, N. sylvestris, N. attenuata, N. undulate, N. otophora, N. suaveolens, N. glauca, N. stocktonii, N. repanda, N. amplexicaulis and N. debneyi* at scaffold or contigs level, chloroplast genomes of five species and mitochondrial genomes of three species are available with one or the other databases. Further, more than two lakh records of gene sequences belonging to 12 *Nicotiana* species are accumulated at various data bases. These databases are sharing the stored information with other databases and providing extensive tools for the analysis of sequences and annotation. INSDC is a long-standing foundational initiative that operates between DNA Databank of Japan (DDBJ), European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) and NCBI and covers the spectrum of data raw reads, through alignments and assemblies to functional annotation. Other databases mentioned above provide access to the *Nicotiana* resources, mostly, through collaboration with other databases along with additional analysis tools existing with their databases. *Nicotiana attenuata* data hub (NaDH) covers the exclusive information on *N. attenuata* and its similarities with other *Nicotiana* species and 11 published dicot species. A website of Boyce Thompson Institute's for *N. benthamiana* resources provide access to *N. benthamiana* genomic resources available at SGN including gene and protein data, markers, genes to phenotypes database etc. (https://btiscience.org/our-research/research-facilities/research-resources/nicotiana-benthamiana). It is also providing tools for alignment, annotation, designing siRNAs for VIGS, CRISPR designing etc.

In addition to above databases, the Gene Ontology resource database provides access to scientific information about the molecular functions of genes (or, more properly, the protein and noncoding RNA molecules produced by genes) from many different organisms, from humans to bacteria, their cellular locations and processes those gene products may carry out (Table 10.6). Currently, 25,761 genes and gene products are found to be associated with the term Nicotiana in The Gene Ontology resource database.

Most of the gene and genomic databases provide tools for searching, alignment and comparison of sequences with other Nicotiana species. Apart from analysis of genome sequence data, various genome databases are facilitating the analysis of gene

Table 10.6 Important genomic resource databases providing information on *Nicotiana*

S. No.	Name	Url
1	National Center for Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov/
2	Sol Genomics Networks (SGN)	http://solgenomics.net/
3	*Nicotiana attenuata* Data Hub (NaDH)	http://nadh.ice.mpg.de
4	Kyoto Encyclopedia of Genes and Genomes (KEGG genome)	https://www.genome.jp/kegg/
5	EnsemblPlants	https://plants.ensembl.org
6	Gramene	https://www.gramene.org/
7	VISTA	https://genome.lbl.gov/
8	The Gene Ontology (GO) Resource	http://geneontology.org/
9	Gene Expression Omnibus (GEO)	https://www.ncbi.nlm.nih.gov/geo/
10	ArrayExpress (AE)	https://www.ebi.ac.uk/arrayexpress/
11	Genomic Expression Archive (GEA)	https://www.ddbj.nig.ac.jp/gea/index-e.html
12	Universal Protein Resource (UniProt)	https://www.uniprot.org/
13	Pfam database	http://pfam.xfam.org/
14	SolCyc Biochemical Pathways	https://solcyc.solgenomics.net/
15	REACTOME	https://reactome.org/
16	The Golm Metabolome Database (GMD)	http://gmd.mpimp-golm.mpg.de/
17	MoNA (Massbank of North America)	https://mona.fiehnlab.ucdavis.edu/
18	Database for Annotation, Visualization, and Integrated Discovery (DAVID)	https://david.ncifcrf.gov/
19	The International Nucleotide Sequence Database Collaboration (INSDC)	https://www.insdc.org/
20	The BioStudies Database	https://www.ebi.ac.uk/biostudies/

variation and expression, analysis and prediction of gene and protein structure and function, prediction and detection of gene regulation networks, etc.

10.13.2 Comparative Genome Databases

The increasing availability of genomic sequence from multiple organisms has provided large dataset for orthologous-sequence comparisons. The rationale for using cross-species sequence comparisons is to identify biologically active regions of a genome based on the observation that sequences that perform important functions are often conserved between evolutionarily distant species, distinguishing them

from nonfunctional surrounding sequences. This is most readily apparent for protein-encoding sequences but also holds true for the sequences involved in the regulation of gene expression.

Comparison of whole-genome sequences at the level of nucleotide or protein provides a detailed narration of syntenic relation at genetic level. Comparative genome studies will identify the types of genes, gene families, and their location also provide information on the history of evolutionary rearrangements of the gene including duplications that might be responsible for the identified genetic variation. By carefully comparing genome characteristics that define various organisms, researchers can pinpoint regions of similarity and difference. This information can be used to identify putative genes and regulatory elements for various traits that may lead to their cloning and further utilization.

A variety of tools for comparison of complete genome sequences of within or between the different species are available in different databases. All the gene and genome databases of tobacco namely NCBI, SGN, NaDH etc. are offering tools for comparative genome analysis. VISTA is a comprehensive suite of programs and databases for comparative analysis of genomic sequences. There are two ways of using VISTA—one can submit their own sequences and alignments for analysis (VISTA servers) or examine pre-computed whole-genome alignments of different species.

Gramene, a knowledge base was founded on comparative functional analyses of genomic and pathway data for model plants and major crops including tobacco. The current release, #64 (September 2021), hosts 114 reference genomes, and round 3.0 million genes from 90 plant genomes with 3,256,006 input proteins in 1,23,064 families with orthologous and paralogous classifications. Comparative genomics collection totals 340 pairwise DNA alignments and 80 synteny maps. Plant Reactome portrays pathway networks using a combination of manual biocuration and orthology-based projections to 106 species. The Reactome platform facilitates comparison between reference and projected pathways, gene expression analyses and overlays of gene–gene interactions. Gramene integrates ontology-based protein structure–function annotation; information on genetic, epigenetic, expression, and phenotypic diversity; and gene functional annotations extracted from plant-focused journals.

Various online/web applications can provides comparative analyses at both the genomic and genic levels tools, such as BRIG (Alikhan et al. 2011), Mauve (Darling et al. 2004), Artemis Comparison Tool (ACT) (Carver et al. 2005), geneCo (Jung et al. 2019) etc. can be used for comparative genomics apart from various tools provided various databases. At *Nicotiana attenuata* data hub, genes of 11 published dicot species were compared and found to cluster into 23,340 homologous groups (HG) based on their sequence similarity with at least two homolog sequences. The phylogenetic trees were also constructed for all these HG.

Comparative analyses of *Nicotiana* plastid genomes among themselves and with currently available Solanaceae genome sequences indicated the existence of similar GC and gene content, codon usage, simple sequence and oligonucleotide repeats, RNA editing sites, and substitutions (Asaf et al. 2016). Such analysis also revealed

that *N. otophora* is a sister species to *N. tomentosiformis* within the *Nicotiana* genus, and *Atropha belladonna* and *Datura stramonium* are their closest relatives (Asaf et al. 2016).

Comparison of whole nuclear and plastid genomes made it possible to identify and confirm of wild progenitor species and their relative genome contributions in the evaluation of cultivated tobacco genomes (Murad et al. 2002; Lim et al. 2004, 2005; Leitch et al. 2008; Sierro et al. 2014, 2018; Edwards et al. 2017). Whole-genomic sequence comparison indicated that the genome of *N. sylvestris* and *N. tomentosiformis* contributes 53 and 47%, respectively, for *N. tabacum* specifying a larger biased genome reduction in T genome (Sierro et al. 2014). In case of *N. rustica*, 41% of genome originated from the paternal donor (*N. undulata*), while 59% originated from the maternal donor (*N. paniculata*/*N. knightiana*) (Sierro et al. 2018) Chloroplast genome comparisons revealed that *N. otophora* is a sister species to *N. tomentosiformis* within *Nicotiana* genus and *Atropha belladonna* and *Datura stramonium* are the closest relatives (Asaf et al. 2016). Maternal parent of the tetraploid *N. rustica* was found to be the common ancestor of *N. paniculata* and *N. knightiana*, and the later species is more closely related to *N. rustica.* Gene clustering analysis revealed the commonality of 14,623 ortholog groups among the *Nicotiana* species and 207 specific to *N. rustica* (Sierro et al. 2018). It was speculated from the results the higher nicotine content of *N. rustica* leaves is the result of the progenitor genomes combination and of a more active transport of nicotine to the shoot.

10.13.3 Gene Expression Databases

Large volume of data is being generated on gene expression patterns in tobacco ranging from seed to senescence under varied conditions in response to abiotic stresses. With initiatives of the Tobacco Genome Initiative (TGI) resulted in enrichment of the sequence information of transcriptionally active regions of the tobacco genome in the form of ESTs, short, single pass sequence reads derived from complementary DNA (cDNA) libraries and methyl filtered genome space sequence reads (GSRs). Kamalay and Goldberg (1980) measured the extent of structural gene expression in an entire tobacco plant. Matsuoka et al. (2004) observed the changes in gene expression during the growth of tobacco BY-2 cell lines and isolated 9,200 EST fragments corresponding to about 7,000 genes. Rushton et al. (2008) identified 2,513 TFs covering the 64 well-characterized plant TF families and these were used to create a database of tobacco transcription factors (TOBFAC). Edwards et al. (2010) designed tobacco expression microarray using Affymetrix platform from a set of 40 k unigenes and measured the gene expression in 19 different tobacco samples to produce the tobacco expression atlas (TobEA). TobEA provides a snapshot of the transcriptional activity of tobacco genes in different tissues throughout the lifecycle of the plant. Expression profiling of tobacco leaf trichomes resulted in the identification of putative genes involved in resistance to biotic and abiotic stresses (Harada et al. 2010; Cui et al. 2011).

The expression databases covers transcript/RNA information of different genes under varied native or test conditions along with the relevant software tools for analysis and retrieval of the data. ESTs is relatively expensive and time consuming. However, Microarrays provide a faster less costly alternative for measuring gene expression simultaneously that can be more easily and reproducibly applied across varied range of conditions to identify genes specific expression patterns or responses. At present the gene expression data have been stored as microarray and RNA-seq datasets in the public databases such as Gene Expression Omnibus (GEO), ArrayExpress (AE) and Genomic Expression Archive (GEA) (Table 10.6). These databases act as useful resources for the functional interpretation of genes and their expression. GEO contained 4,860 curated gene expression data sets as well as original series and platform records of 11 *Nicotiana* spp. (https://www.ncbi.nlm.nih.gov/gds). Genomic Expression Archive has 205 gene expression records of 11 *Nicotiana* spp. SGN is maintaining 39 transcript libraries of *N. tabacum* and two of *N. sylvestris*. Further, there are exclusive expression databases for *Nicotiana attenuata* (NaDH) and *N. benthamiana* (https://btiscience.org/our-research/research-facilities/research-resources/nicotiana-benthamiana) along with the Sol genome networks for expression analysis among solanaceous members.

10.13.4 Protein or Metabolome Databases

Proteome is the study of proteins thought to be expressed by an organism in its life cycle. The metabolome deals with the metabolites of small size (<1500 Da) in a specific cell of an organ or organism. The metabolome of the plant act as link between genotype and phenotype. It also indicates the stage/organ specific response of the plants through gene expression in response to external environment. It not only influences the gene expression but also affects the protein functions of the plant which make metabolomics a central component in elucidating cellular systems and decoding gene functions.

Proteomics and metabolomics approaches play significant role in functional genomics and have been essentially required for understanding plant development and abiotic stress tolerance. Proteome and metabolome profiling are potential tool for phenotyping plants under varied environmental changes and biotic stresses. Such studies contribute significantly to the study of abiotic stress biology by distinguishing different compounds such as auxiliary products of stress metabolism from biosynthetically complex abiotic pathways, stress induced signal molecules, molecules that are part of plant acclimation process etc.

The resultant metabolic compounds could be further studied by direct measurement or correlating with the changes in transcriptome and proteome expression during stress condition and can be confirmed by mutant analysis. Thus, metabolome study aid in unravelling the different pathways related to plant development and response to stresses. With the advent of high throughput-based systems, proteome and metabolome profiling was extensively carried out in the model plant like tobacco to

examine stress signaling pathways, cellular and developmental processes (Xiang et al. 2016). Principal databases hosting tobacco proteome information are UniProt, Pfam, KEGG, SGN, NCBI, etc. and metabolome databases are SolCyc, REACTOME, The Golm Metabolome Database (GMD), MoNA (Massbank of North America), etc. The salient features of these data bases are briefed below.

UniProt, an association between the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB), and the Protein Information Resource (PIR), is comprised of three databases, each optimized for different uses. Universal Protein Resource (UniProt), a comprehensive resource for protein sequence and annotation data base generated globally from proteomic data. The UniProt Knowledge base (UniProtKB) is the central access point for extensively curated protein information, function, classification and cross-references. The UniProt Reference Clusters (UniRef) combine closely related sequences into a single record to speed up sequence similarity searches. The UniProt Archive (UniParc) is a comprehensive repository of all protein sequences, consisting only of unique identifiers and sequences. UniProt provides several sets of proteins thought to be expressed by organisms whose genomes have been completely sequenced, termed "proteomes". There are 73,606 protein entries associated with proteome *N. tabacum* and a total of 154,728 entries for all *Nicotiana* species as on 30.09.2021. The Pfam database is a large collection of protein families, each represented by multiple sequence alignments. This database provides tools for protein alignments and annotation, domain organization of a protein sequence etc. There are about 4,970 unique results for the search term *Nicotiana* in this data base as on 30.09.2021 indicating the protein entries in the database. KEGG database in addition to providing protein information, projects the biological processes from various organisms onto pathways consolidated into large network schemes. At present, the KEGG database have the information of annotated proteins of *N. tabacum* (61,780 No.), *N. tomentosiformis* (30,989) *N. sylvestris* (33,816) and *N. attenuata* (34,218). In the Sol genomics network database also providing the data of proteins annotated based on the draft genome sequences of *N. tabacum, N. benthamiana* and *N. attenuata.* Around 275,000 collection of protein sequences from several sources are available for 20 *Nicotiana* spp. at NCBI along with annotated reports of four *Nicotiana* spp. (Table 10.4). SolCyc is a collection of Pathway Genome Databases (PGDBs) for Solanaceae species generated using Pathway Tools. It is a database hub at SGN for the manual curation of metabolic networks and includes annotated metabolic, regulatory and signaling processes in Solanaceous plants based on Omics data obtained from multiple resources. It has species-specific databases for *N. tabacum* (K326Cyc), *N. attenuata* (NattCyc), *N. sylvestris* (NiSylCyc), *N. tomentosiformis* (NiTomCyc), *N. benthamiana* (BenthaCyc); and multi-species databases for Combined *Nicotiana* genus (Nicotiana Cyc) and Combined Solanaceae database (Solana Cyc). Apart from the proteomic data, NaDH is providing metabolome database with analysis tools for *N. attenuata* with facilities for the search of metabolites and fragments based on annotation and measured values.

REACTOME offers bioinformatics tools for the visualization, interpretation and analysis of pathway knowledge to support basic and clinical research, genome analysis, modeling, systems biology and education. GMD, an open access metabolome database provides public access to custom mass spectral libraries, metabolite profiling experiments as well as additional information and tools, e.g. with regard to methods, spectral information or compounds. MoNA (Massbank of North America) is a centralized and collaborative database of metabolite mass spectra, metadata and associated compounds. MoNA currently contains over 200,000 mass spectral records from experimental and in-silico libraries as well as from user contributions.

The proteomic studies in tobacco revealed different stress responses (Amme et al. 2005). Analysis of the proteome of glandular trichomes revealed the enrichment of proteins belonging to components of stress defense responses. Metabolome study under water stress in tobacco identified a useful marker for drought stress for members of Solanaceae (Rabara et al. 2017).

10.13.5 Integration of Different Data

Analysis of omics data provides biological understanding at a specific molecular layer such as genome, proteome, transcriptome and metabolome. Understanding of agronomic traits requires the complete knowledge of complex crosstalk between different molecular layers, such as genome, proteome, transcriptome and metabolome. An integrative analysis of multiple layers of molecular data or system biology helps to discover and elucidate molecular mechanisms of phenotypic traits and resistance responses to various abiotic stresses (Singh et al. 2016). With the advent of high-throughput techniques and availability of multi-omics data generated from a large set of samples, lot of promising tools and methods have been developed for data integration and interpretation. Most of the biological databases collects and integrate data from different sources.

Databases namely INSDC, NCBI, SGN, NaDH, KEGG genome, EnsemblPlants, DAVID (Database for Annotation, Visualization, and Integrated Discovery), The BioStudies Database etc. are some of the integrated databases and resources that are collecting and integrating the omics data from different plants and tobacco (Table 10.6). As a collaborative foundational initiative, INSDC covers the spectrum of data raw reads, through alignments and assemblies to functional annotation, enriched with contextual information relating to samples and experimental configurations. NCBI in addition to providing wide-ranging tobacco data information in its different databases, offers tools for integration of structural and functional genomic data and their annotations. The curated proteome data of *Nicotiana* species in Uniport, and metabolome data from different resources are being integrated in new datahubs like SGN, KEGG, NaDH etc. to provide holistic information from gene to pathway for the researchers. KEGG is an integrated resource database consisting of 16 databases including genes and proteins, metabolites and other chemical substances, biochemical reactions, enzyme, disease-related network variations etc.

EnsemblPlants is an integrative resource that includes genome-scale information for sequenced plant species (currently 33 in no.). Data provided includes genome sequence, gene models, functional annotation, and polymorphic loci. DAVID is a web-accessible database that integrates functional genomic annotations with intuitive graphical summaries. Lists of gene or protein identifiers are rapidly annotated and summarized according to shared categorical data for gene ontology, protein domain, and biochemical pathway membership. Numerous public sources of protein and gene annotation information have been integrated into DAVID database for over 1.5 million genes from more than 65,000 species. European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) is building BioStudies Database, a resource for accepting and archiving data generated in "multi-omics" experiments. Building of archives, databases and analysis tools in an integrated approach have proven successful for better understanding and comparing omic resources of tobacco.

10.14 Brief Account on Social, Political and Regulatory Issues

10.14.1 Concerns and Compliances

Tobacco is high value commercial crop generating higher farm income and revenue to national government and farmers. The crop is providing livelihood security to the people involved in tobacco production, processing and marketing in one side and on the other side it is posing serious health risks to consumers. In another way use of huge quantity of forest wood for FCV curing causing deforestation, smoking related environment pollution and also spitting habits of chewing tobacco are causes for concern. Climate change patterns, emerging biotic and abiotic stresses, pesticide residues, consumer preferences and tobacco regulatory policies are becoming increasingly complex and posing challenges for tobacco cultivation (ICAR 2015). WHO-Framework Convention on Tobacco Control (FCTC), with membership of 182 countries, envisages non-price, price and tax measures to reduce the supply and demand for tobacco in the world. On May 31st every year, the world observes World No Tobacco Day (WNTD) promoted by the World Health Organization (WHO) with a primary focus on encouraging users to refrain from tobacco consumption and its related products at least for a period of 24 h.

10.14.2 Patent and IPR Issues

Researchers are developing various management strategies for minimizing crop yield losses at field level due to abiotic stresses. This includes development of abiotic stress

tolerant varieties through conventional breeding and biotechnological interventions. The advances in biotechnology and bioinformatics generated various genome-based tools, techniques, genes and gene constructs in the field of abiotic stress tolerance (Dangl and Jones 2001). Intellectual property rights (IPR) for plants help protect inventions made in research and development of new tobacco varieties (CORESTA 2005). In turn, this encourages investment and helps continue the development of new varieties that increases economic returns throughout the tobacco supply chain. Over the past 25 years, an increasing number of governments and international organizations have enacted laws, regulations, or policies that acknowledge the need for IPR.

Patents provides the ownership right to make, use, sell, offer for sale, or import for those purposes a patented product. The ability to patent plant varieties is recognized in some countries, but in many countries such as India it is disallowed. Instead India provided farmers rights considering their historical contribution of preserving and protecting valuable genetic resources. There have been few patents granted for tobacco varieties in the world (CORESTA 2005). United States permitted the use of utility patents to protect plant varieties. European countries that are members of the European Patent Office (EPO) ban the patenting of plant varieties, but recent determinations support patent claims directed to plants of more than one variety. Thus, a utility patent and the ruling by the EPO may suggest a type of broad protection for a novel plant trait that is not recognized under PVP.

Recent advances in molecular biology, plant genomics and crop science have brought about a paradigm shift of thought regarding how tobacco plants can be utilized for commercial and medicinal uses. There is a scope for patenting of novel methods of making abiotic stress resistant tobacco genotypes, methods of introgressing nucleic acid molecules associated with stresses, genes conferring resistance to various stresses (Hefferon 2010). Patenting activity in resistance genes in tobacco is initiated in 1992 and there is considerable progress in patenting from the year 2000 and it was more prominent from 2010 onwards (Prabhakararao et al. 2016). Majority of these patent documents (around 60%) are in the jurisdiction of United States of America (USA) and China.

The intellectual information generated in the frontier areas is available in both non-patent and patent literature. Nearly 80% of all the technical information available in the world is hidden in the patent documents and other IP assets (Prabhakararao et al. 2016). Patent mapping helps in retrieving and exploring the information protected in the intellectual property documents. Collections of patent documents are available in a number of patent information databases (https://guides.library.queensu.ca/patents/databases). Most patent offices provide free access to patent documents via public databases. Patent information can be used to decide the patentability of an invention, avoid re-invention and infringement, provide the current state of the art in a given field of technology, find the latest trends in R and D being pursued by the peers and competitors etc.

10.14.3 Disclosure of Sources of GRs, Access and Benefit Sharing

Genetic resources (GRs) are a key for the number of biotechnological innovations (Steward 2018). History reveals that less than 1% of species have provided the basic resources for the development of all civilization. It is not possible to predict which genes, species or ecosystems will become valuable in the future. Over the last decades, regulations have been developed that aim to improve the sustainable use of GRs and benefit sharing i.e., Access-Benefit Sharing (ABS). Convention on Biological Diversity (CBD) adopted in 1992 serves as initiation point in many countries for biodiversity conservation and use. The more recent Nagoya protocol, a 2010 supplementary agreement to the CBD, aims to improve the fair and equitable sharing of benefits arising out of the utilization of genetic resources. The existing ABS systems vary widely and GR-rich countries tending to organize their systems more strictly focusing on acquiring an equitable share of the benefits related to products resulting from the use of GR. Over the years, several governments introduced disclosure requirements (DRs) in the patent system as an extra component to enhance ABS compliance.

10.14.4 Framers' Rights

Farmers around the world have been the custodians, innovators and protectors of agricultural biodiversity (Craig 2004; FAO 2017) since the dawn of cultivation of crop plants. Farmers are involved in collecting the best seeds and cultivating various types of tobacco species/types throughout the world. Through the careful selection of their best seeds, propagation of material, and exchange with each other farmers lead to develop of new innovations/varieties as well as to diversify crop varieties. Country like India, households traditionally raising different tobacco landraces in their kitchen gardens since generations from the seeds collected from their own crops, thus maintaining and protecting biodiversity. Considering the past, present and future contributions of farmers in conserving, improving, and making available plant genetic resources, farmer's rights are to be protected. Farmers' access to use and exchange the seed and propagating material are to be protected from seed regulations (variety release and seed marketing regulations), legislation concerning to intellectual property rights (patents and plant breeders' rights), and regulations dealing with bio-prospecting of genetic resources.

The concept of farmers' rights was emerged in international negotiations within FAO in 1986 to counter the increased demands for plant breeders' rights (PBR) being voiced in international negotiations. In 1987, solutions were being proposed, serving as the foundation for all further negotiations on Farmers' Rights. In 1989, farmers' Rights gained formal recognition by the FAO Conference. In 1991, the Conference

decided to set up a fund for the realization of these rights, but this has never materialized. CBD with its resolution on the promotion of sustainable agriculture urged FAO to commence negotiations for a legally binding international regime on the management of plant genetic resources and to resolve the question of Farmers' Rights. Agenda 21 approved at the UN Conference on Environment and Development held in Rio de Janeiro in 1991 had voiced similar demands. In November 1996, Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (Global Plan of Action) acknowledges the need to realize Farmers' Rights and was endorsed by the FAO Council, by the Conference of the Parties to the CBD, and by the World Food Summit at FAO. The Second Global Plan of Action prepared under the aegis of the Commission on Genetic Resources for Food and Agriculture was adopted by the FAO Council in 2011. This action plan contains a set of recommendations and activities intended as a framework, guide and catalyst for action at community, national, regional and international levels.

The International Treaty on Plant Genetic Resources for Food and Agriculture (International Treaty) adopted in 2001 addressed the issue of Farmers' Rights in its Preamble and Article 9. The International Treaty recommends Contracting Parties to protect and promote Farmers' Rights in accordance with their national laws. In Article 9, the Treaty recognize the enormous contribution made and will continue to make by the farmers of all regions of the world for the conservation and development of plant genetic resources as the basis of food and agricultural production throughout the world. Measures are suggested that covering the protection of traditional knowledge, benefit-sharing and participation in decision-making. The rights of farmers to save, use, exchange and sell farm-saved seeds and propagating material are addressed in the International Treaty, but without any legally binding provisions on how to implement Farmers' Rights at national level.

10.14.5 Traditional Knowledge

Local communities uses traditional knowledge (TK) generated using long standing traditions and practices in coping with extreme weather and adapting to climate change from the centuries for their survival (Swiderska et al. 2011). The diversity of traditional varieties maintained by farmers around the world are important for adaptation to climate changes and emerging issues of abiotic stresses. Local communities use wild foods to supplement their diets and thus conserve wild species which are valuable sources of abiotic stress resistant genes. The traditional varieties or landraces are genetically more diverse than modern varieties and are good sources of resistance to abiotic stresses (https://www.cbd.int/traditional/what.shtml). Because of their long experience in cultivating crops under varied changing climates, the traditional farmers are well placed to identify resilient crop species and resistant varieties for abiotic stresses with the available accumulated TK. Traditional Knowledge about resilient properties, such as abiotic and biotic stress resistance traits and wild crop relatives

can be a valuable information for developing stress tolerant tobacco varieties (Jarvis et al. 2008).

10.14.6 Treaties and Conventions

International agreements that have special significance in the context of agricultural sector in general and biotechnology particular are CBD, International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA) and The International Union for the Protection of New Varieties of Plants (UPOV). The salient features and provisions of them are briefly discussed hereunder.

10.14.6.1 Convention on Biological Diversity (CBD)

The Convention on Biological Diversity (CBD), widely known as the Biodiversity Convention, is an international legally binding treaty and was ratified in 1992 at the Rio earth summit (https://www.cbd.int/convention/). It is often seen as the key document concerning the sustainable development and currently has 196 Parties (168 Signatures). The Convention has three main goals viz., conservation of biological diversity (or biodiversity), sustainable use of its components and fair and equitable sharing of benefits arising from genetic resources. The convention recognized for the first time in international law that the conservation of biological diversity is "a common concern of humankind". It is an integral part of the development process and covers all ecosystems, species, and genetic resources. The objective of CBD is to develop national strategies for the conservation and sustainable use of biological diversity. The Contracting Parties shall, in accordance with national legislation and policies, encourage and develop methods of cooperation for the development and use of technologies, including indigenous and traditional technologies, in pursuance of the objectives of this Convention. It links traditional conservation efforts to the economic goal of using biological resources sustainably. The Contracting Parties shall promote international technical and scientific cooperation in the area of conservation and sustainable use of biological diversity. For this purpose, the Contracting Parties shall also promote cooperation in the training of personnel and exchange of experts. Article 19 of the Convention deals with the issues in respect of handling of living modified organism resulting from biotechnology.

CBD has three important protocols viz., The Nagoya Protocol on Access and Benefit-sharing, The Cartagena Protocol on Biosafety and The Nagoya—Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety under CBD. The essential features of these protocols are briefed below.

10.14.6.2 Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety is an international treaty governing the movements of living modified organisms (LMOs) resulting from modern biotechnology from one country to another (http://bch.cbd.int/protocol). Adopted as a supplementary agreement to CBD, it came into force on 11 September 2003. The objective of the Protocol is to ensure an adequate level of protection in the field of the safe transfer, handling and use of 'living modified organisms resulting from modern biotechnology' that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on trans-boundary movements. The Protocol provides for Parties to enter into bilateral, regional and multilateral agreements and arrangements regarding international trans-boundary movements of living modified organisms.

The Protocol establishes a Bio-Safety Clearing-House to: (a) Facilitate the exchange of scientific, technical, environmental and legal information on, and experience with, living modified organisms; and, (b) Assist Parties to implement the Protocol, taking into account the special needs of developing country Parties, in particular the least developed and small island developing States among them, and countries with economies in transition as well as countries that are centres of origin and centres of genetic diversity.

10.14.6.3 Nagoya Protocol

The Nagoya Protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their Utilization (ABS, is a supplementary international agreement to CBD, adopted on 29 October 2010 in Nagoya, Japan and entered into force on 12 October 2014 (https://www.cbd.int/abs/). Currently, it has 131 Parties (132 ratifications) (92 signatories). The protocol provides for a transparent legal framework for the effective implementation of fair and equitable sharing of benefits arising out of the utilization of genetic resources. It applies mainly to genetic resources and traditional knowledge (TK) associated with genetic resources that are covered under CBD, and to the benefits arising from their utilization.

The Nagoya—Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety

Adopted as a supplementary agreement to the Cartagena Protocol, it aims to contribute to the conservation and sustainable use of biodiversity by providing international rules and procedures in the field of liability and redress relating to living modified organisms (http://bch.cbd.int/protocol/supplementary/). The Protocol was entered into force on 5 March 2018 and currently, it has 49 Parties. The Protocol requires that response measures are taken in the event of damage resulting from living modified organisms which find their origin in a transboundary movement, or where

there is sufficient likelihood that damage will result if timely response measures are not taken. A definition of 'damage', referring to an adverse effect on the conservation and sustainable use of biological diversity that is measurable or otherwise observable and significant, taking also into account risks to human health is provided in the Protocol. The Protocol necessitates a causal link between the damage and the living modified organism be established. While imposing the requirement for response measures, the Protocol obliges the Parties to continue to apply existing legislation on civil liability or to develop specific legislation concerning liability for material or personal damage associated with the conservation and sustainable use of biological diversity.

10.14.6.4 The International Treaty on Plant Genetic Resources for Food and Agriculture

The International Treaty on Plant Genetic Resources for Food and Agriculture Adopted in 2001 (FAO 2009) with the objectives of conservation and sustainable use of all plant genetic resources for food and agriculture and the fair and equitable sharing of the benefits arising out of their use, in harmony with CBD, for sustainable agriculture and food security. This legally binding Treaty covers all plant genetic resources and vital in ensuring the continued availability of the plant genetic resources that countries will need to feed their people. The Treaty recognizes the enormous contribution made by the local and indigenous communities and farmers of all regions of the world and takes measures for protecting Farmers' Rights. The Contracting Parties agree to establish an efficient, effective, and transparent multilateral system to facilitate access to plant genetic resources for food and agriculture, and to share, in a fair and equitable way, the benefits arising from their utilization. The treaty takes care of (a) protection of traditional knowledge; (b) the right to equitably participate in sharing benefits arising from the utilization of plant genetic resources; and (c) the right to participate in making decisions, at the national level, on matters related to the conservation and sustainable use of plant genetic resources for food and agriculture.

10.14.6.5 The UPOV Convention

The UPOV (International Union for Protection of Plant Varieties) Convention was adopted for the protection of plant variety in Paris in 1961 and entered into force in 1968. It was subsequently revised in 1972, 1978 and 1991. The 1978 Act entered into force in 1981, and the 1991 Act in 1998 (www.upov.int). An inter-governmental organization was established with headquarters in Geneva, Switzerland. UPOV's mission is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society. Plant Variety Protection (PVP) under UPOV is enabled in the 77 members countries. The current act of the convention adopted in 1991 recognizes breeder's

rights to a variety if the variety is: (1) new; (2) distinct; (3) uniform; and (4) stable. The breeder's rights as per 1991 Act require authorization of the breeder to perform the following: (1) production or reproduction (multiplication) (2) conditioning for the purpose of propagation, (3) offering for sale, (4) selling or marketing, (5) exporting, (6) importing, (7) stocking for any purpose mentioned in (1) to (6) above. Breeder's rights to a variety remain in effect for a period of 20 years from the date on which the rights were granted. The Act of 1991, for the first time included the protection against "essentially derived" varieties, which are derived from the protected variety, that is not clearly distinguishable from the protected variety, or which requires repeated use of the protected variety for production purposes. An essentially derived variety could be developed from a protected variety through: (1) the selection of a natural or induced mutant, or of a somaclonal variant, (2) the selection of a variant individual from that of the initial variety, (3) backcrossing, and (4) transformation by genetic engineering. Exceptions to breeder's rights were granted for: (1) acts done privately and for non-commercial purposes, (2) acts done for experimental purposes, and (3) acts done for the purpose of breeding other varieties, except for the generation of essentially derived varieties.

10.14.7 Participatory Breeding

For thousands of years prior to 1800s, during and after the domestication of *Nicotiana* species, one of the principal method of tobacco improvement was through conservation of diversity and selection of naturally occurring high yielding and stress resistant variants by cultivators. The systematic varietal improvement started by scientists in 1990s in established research organizations globally has led to release of number of high yielding stress resistant tobacco varieties through conventional plant breeding techniques.

In view of the limitations to formal breeding and the threats to farmers' seed systems, participatory plant breeding (PPB) emerged as a means to overcome some of the limitations of formal system and to bring farmers back into the breeding process as active participants (Greenberg 2018). The role played by farmers in agricultural biodiversity conservation and use is taken as an advantage while making them as important partners in breeding plant varieties. In the development of improved varieties, PPB ensures the improvement of the adapted local genetic materials using the diversity available either with them or public gene banks to suit the farmer needs prevailing under their conditions. This also empower the farmer in terms of technical and organizational skills in maintaining and developing plant materials under their control, their on-farm management, and local creativity/innovation. PPB involves the active participation of farmers in few or all the steps of sequenced breeding program viz., priority setting, production and sharing of genetic materials and knowledge, acquisition of genetic material and selection, crossing, selection at early/advanced stages, and evaluation. PPB a complementary breeding process and may not be a

substitute for station-based research or scientist-managed on-farm trials (Hardon et al. 2005; Aguilar-Espinoza 2007; Ceccarelli et al. 2009).

10.15 Future Perspectives

10.15.1 Potential for Expansion of Productivity

The improvements in crop yield through conventional approaches is still achievable in tobacco (Sarala et al. 2016). The genotype improvements in tobacco for increasing and stabilizing yields can further be accelerated through the combination of traditional breeding techniques with genome designing strategies. Advancements in genomic research would assist in designing of appropriate genome assisted breeding strategies for attaining maximum potential yields with good quality and required stress resistance in a short span of time.

10.15.2 Potential for Expansion into Nontraditional Areas

Tobacco is an important commercial crop that plays a significant role in the economies of many countries (FAO 2019). Another way, it is one of the most important model systems in plant biotechnology till date and going to continue further. *Nicotiana* species are investigated for aspects concerning the elucidation of principles of disease resistance, synthesis of secondary metabolites and basic questions of plant physiology. In view of its higher level of biomass accumulation, tobacco is a promising crop to produce commercially important substances (e.g., antigens, antibodies, drugs and vaccines) through molecular farming and cultivation of tobacco for its valuable native phyto-chemicals viz., nicotine, solanesol, proteins and organic acids (Sarala 2019). Hence, tailoring tobacco for molecular farming is going to be an important objective for tobacco improvement programs.

References

Abdurakhmonov IY, Abdukarimov A (2008) Application of association mapping to understanding the genetic diversity of plant germplasm resources. Intl J Plant Genom. https://doi.org/10.1155/2008/574927

Abrams MD, Kubiske ME Steiner KC (1990) Drought adaptation and responses in five genotypes of *Fraxinus pennsylvanica* Marsh.: photosynthesis, water relations and leaf morphology. Tree Physiol 6:305–315

Aguilar-Espinoza E (2007) Evaluating a participatory plant breeding approach in a local seed system of Northern Nicaragua: challenges for upscaling technologies and institutions. Master's thesis, Norwegian University of Life Sciences

Ahmar S, Mahmood T, Fiaz S et al (2021) Advantage of nanotechnology-based genome editing system and its application in crop improvement. Front Plant Sci 12:663849. https://doi.org/10.3389/fpls.2021.663849

Ajab H, Yaqub A, Malik SA et al (2014) Characterization of toxic metals in tobacco smoke and cigarette ash from selected imported and local brands in Pakistan. Sci World J. https://doi.org/10.1155/2014/413614D

Akehurst BC (1981) Tropical agricultural series tobacco, 2nd edn. New York

Ali MA, Abbas A, Niaz S et al (2009) Morpho-physiological criteria for drought tolerance in sorghum (Sorghum bicolor) at seedling and post-anthesis stages. Intl J Agri Biol 11:674–680

Ali Z, Abul-Faraj A, Piatek M et al (2015) Activity and specificity of TRV-mediated gene editing in plants. Plant Signal Behav 10(10):e1044191. https://doi.org/10.1080/15592324.2015.1044191

Alikhan NF, Petty NK, Zakour NLB et al (2011) BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402

Amme S, Rutten T, Melzer M et al (2005) A proteome approach defines protective functions of tobacco leaf trichomes. Proteomics 5(10):2508–2518. https://doi.org/10.1002/pmic.200401274

Anderson E, Dorothea DW (1931) The genetic analysis of an unusual relationship between self-sterility and self-fertility in *Nicotiana*. Ann MO Bot Gard 18(1):97–116. https://doi.org/10.2307/2394046

Anderson J, Wagner M, Rushworth C et al (2014) The evolution of quantitative traits in complex environments. Heredity 112:4–12. https://doi.org/10.1038/hdy.2013.33

Anuradha M, Sivaraju K, Krishnamurthy V (2013) Effect of waterlogging on physiological characteristics, yield and quality of flue-cured tobacco. Indian J Plant Physiol 18:67–70

Arslan B, Okumus A (2006) Genetic and geographic polymorphism of cultivated tobaccos (*Nicotiana tabacum*) in Turkey. Genetika 42(6):818–823. https://doi.org/10.1134/S1022795406060123

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Asaf S, Khan AL, Khan AR et al (2016) Complete chloroplast genome of *Nicotiana otophora* and its comparison with related species. Front Plant Sci 7:843. https://doi.org/10.3389/fpls.2016.00843

Baghyalakshmi K, Sarala K, Prabhakara Rao K et al (2018) Genetic diversity in tobacco genotypes evaluated under All India Network Project. Multilogic Sci 8 (issue special):30–34

Bailey JE (1991) Towards a science of metabolic engineering. Science 21:252(5013):1668–1675. https://doi.org/10.1126/science.2047876. PMID: 2047876

Bakhsh A, Dinç T, Hussain T (2018) Development of transgenic tobacco lines with pyramided insect resistant genes. Turk J Biol 42(2):174–186. https://doi.org/10.3906/biy-1710-71

Baloglu MC, Oz MT, Oktem HA et al (2012) Expression analysis of TaNAC69-1 and TtNAMB-2, Wheat NAC family transcription factor genes under abiotic stress conditions in durum wheat (*Triticum turgidum*). Plant Mol Biol Rep 30:1246–1252

Barchi L, Bonnet J, Boudet C et al (2007) A high-resolution, intraspecific linkage map of pepper (*Capsicum annuum* L.) and selection of reduced recombinant inbred line subsets for fast mapping. Genome 50(1):51–60

Barnes JD, Kevin E, Paul ND et al (1996) The influence of UV-B radiation on the physicochemical nature of tobacco (*Nicotiana tabacum* L.) leaf surfaces. J Exp Bot 47(1):99–109

Barrett BA, Kidwell KK (1998) AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci 38:1261–1271

Basirnia A, Hatami MH, Darvishzadeh R et al (2014) Mixed linear model association mapping for low chloride accumulation rate in oriental-type tobacco. J Plant Interact 9:666–672

Beavis WD (1998) QTL analysis: power, precision, and accuracy. In: Paterson A (ed) Molecular dissection of complex traits. CRC Press, Boca Raton, Florida

Belknap WR, Garbarino JE (1996) The role of ubiquitin in plant senescence and stress response. Trends Plant Sci 1:331–335

Berbec A, Doroszewska T (2020) The use of *Nicotiana* species in tobacco improvement. In: Ivanov NV, Sierro N, Peitsch MC (eds) The tobacco plant genome, compendium of plant genomes. Springer Nature, Switzerland AG. https://doi.org/10.1007/978-3-030-29493-9_8
Binbin H, Geng R, Cheng L et al (2020) Genetic diversity and fingerprinting of 33 standard flue-cured tobacco varieties for use in distinctness, uniformity and stability testing. BMC Plant Biol 20:378
Bindler G, Plieske J, Bakaher N et al (2011) A high density genetic map of tobacco (*Nicotiana tabacum* L.) obtained from large-scale microsatellite marker development. Theor Appl Genet 123:219–230
Bindler G, van der Hoeven R, Gunduz I et al (2007) A microsatellite marker-based linkage map of tobacco. Theor Appl Genet 114:341–349
Bliss LC, Kramer PJ, Wolf FA (1957) Drought Resistance in Tobacco. Tob Sci 1–29:120–123
Boccardo NA, Segretin ME, Hernandez I et al (2019) Expression of pathogenesis-related proteins in transplastomic tobacco plants confers resistance to filamentous pathogens under field trials. Sci Rep 9:2791. https://doi.org/10.1038/s41598-019-39568-6
Bock R (2013) Strategies for metabolic pathway engineering with multiple transgenes. Plant Mol Biol 83(1–2):21–31. https://doi.org/10.1007/s11103-013-0045-0
Boursiac Y, Léran S, Corratgé-Faillie C et al (2013) ABA transport and transporters. Trends Plant Sci 18(6):325–333
Bovy A, de Vos R, Kemper M et al (2002) High-flavonol tomatoes resulting from the heterologous expression of the maize transcription factor genes LC and C1. Plant Cell 14:2509–2526
Bowman DT, Sisson V (2000) A historical overview of flue-cured tobacco breeding in the U.S.A. Tob Sci 44:59–64. https://doi.org/10.3381/0082-4623-44.1.59
Bradbury PJ, Zhang Z, Kroon DE et al (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23(19):2633–2635
Bretting PK, Widrlechner MP (1995) Genetic markers and plant genetic resource management. Plant Breed Rev 13:11–86
Brieger FG (1935) Genetic analysis of the cross between self-fertile *N. Langsdorfii* and the self-sterile *N. Sanderae*. J Genet 30(1):79–100
Broun P (2004) Transcription factors as tools for metabolic engineering in plants. Curr Opin Plant Biol 2:202–209
Brown JKM (2002) Yield penalties of disease resistance in crops. Curr Opin Plant Biol 5:339–344
Bruner WB (1931) Root development of cotton, peanuts and tobacco in central Oklahoma. Proc Oklahoma Acad Sci 1931:20–37
Burbridge TN (1960) The Australian species of *Nicotiana* L. (Solanaceae). Aust J Bot 8:342–380
Burklew CE, Ashlock J, Winfrey WB et al (2012) Effects of Aluminum oxide Nanoparticles on the growth, development and microRNA expression of Tobacco (*Nicotiana tabacum*). PLoS One 7(5):e34783. https://doi.org/10.1371/journal.pone.0034783
Burlaka OM, Pirko YV, Yemets AI et al (2015) Plant genetic transformation using carbon nanotubes for DNA delivery. Cytol Genet 49:3–12. https://doi.org/10.3103/S009545271506002X
Buschmann C, Lichtenthaler HK (1998) Principles and characteristics of multi-colour fluorescence imaging of plants. J Plant Physiol 152:297–314. https://doi.org/10.1016/s0176-1617(98)80144-2
Butow RA, Fox TD (1990) Organelle transformation: shoot first, ask questions later. Trends Biochem Sci 15(12):465–468. https://doi.org/10.1016/0968-0004(90)90299-Q
Cai C, Yang Y, Cheng L et al (2015) Development and assessment of EST-SSR marker for the genetic diversity among tobaccos (*Nicotiana tabacum* L.) Russ J Genet 51:591–600
Cai CC, Chai LG, Wang Y et al (2009) Construction of genetic linkage map of burley tobacco (*Nicotiana tabacum* L.) and genetic dissection of partial traits. Acta Agron Sin 35:1646–1654
Cameron KD, Teece MA, Smart LB (2006) Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiol 140:176–183. https://doi.org/10.1104/pp.105.069724
Campbell RB (1973) Flue cured tobacco yield and oxygen content of soil in lysimeters flooded for various periods. Agron J 65:783–786

Carver TJ, Rutherford KM, Berriman M et al (2005) ACT: the Artemis comparison tool. Bioinformatics 21(16):3422–3423. https://doi.org/10.1093/bioinformatics/bti553
Ceccarelli S, Guimaraes E, Weltzien E (eds) (2009) Plant breeding and farmer participation. FAO, Rome
Champoux MC, Wang G, Sarkarung S et al (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet 90:969–981
Chaplin JF, Mann TJ (1978) Evaluation of tobacco mosaic resistance factor transferred from burley to flue-cured tobacco. J Hered 69:175–178
Chaplin JF, Matzinger DF, Mann TJ (1966) Influence of homozygous and heterozygous mosaic-resistance factor on quantitative characters of flue-cured tobacco. Tob Sci 10:81–84
Chari MS (1995) Role of research in the improvement of productivity and quality of Indian flue-cured Virginia tobacco. Central Tobacco Research Institute, Rajahmundry, India, p 26
Chase MW, Knapp S, Cox AV et al (2003) Molecular systematics, GISH and the origin of hybrid taxa in Nicotiana (Solanaceae). Ann Bot 92:107–127
Cheng L, Chen X, Jiang C et al (2019) High-density SNP genetic linkage map construction and quantitative trait locus mapping for resistance to cucumber mosaic virus in tobacco (*Nicotiana tabacum* L.). Crop J 7(4):539–547. https://doi.org/10.1016/j.cj.2018.11.010
Chéour F, Arul J, Makhlouf J et al (1992) Delay of membrane lipid degradation by calcium treatment during cabbage leaf senescence. Plant Physiol 4:1656–1660
Chuanyin D, Zhizhong H, Xuliang Y (2009) AFLP analysis of genetic diversity of tobacco germplasms. Paper presented at 63rd tobacco science research conference, Florida, 27–30 Sept 2009
Clark JM, Townley-Smith TF, McCaig TN et al (1984) Growth analysis of spring wheat cultivars of varying drought resistance. Crop Sci 24:537–541
Clarkson JJ, Knapp S, Garcia VF et al (2004) Phylogenetic relationships in *Nicotiana* (*Solanaceae*) inferred from multiple plastid DNA regions. Mol Phylogenet Evol 33:75–90
Clarkson JJ, Lim KY, Kovarik A et al (2005) Long-term genome diploidization in allopolyploid *Nicotiana* section Repandae (Solanaceae). New Phytol 168:241–252
Clausen RE, Cameron DR (1944) Inheritance in *Nicotiana tabacum* Xviii. Monosomic analysis. Genetics 29(5):447–477
Clausen RE, Goodspeed TH (1926) Inheritance in *Nicotiana tabacum* VII. The monosomic character, fluted. Univ Calif Pub Bot 11:61–82
Cocucci M, Negrini N (1988) Changes in the levels of calmodulin and of a calmodulin inhibitor in the early phases of radish (*Raphanus sativus* L.) seed germination—effects of ABA and fusicoccin. Plant Physiol 88:910–914
Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Phil Trans Roy Soc Lond B Biol Sci 363:557–572
CORESTA (2005) Tobacco seed integrity task force final report—May 2005, 1–14 pp. https://www.coresta.org/sites/default/files/technical_documents/main/Seed-Integrity-TF-May05.pdf. Accessed 13 Jul 2021
Craig B (2004) Farmers' rights: intellectual property regimes and the struggle over seeds. Politics Society 32(4):511–543. https://doi.org/10.1177/0032329204269979
Cramer GR, Nowak RS (1992) Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley. Physiol Planta 84:600–605
Cui H, Zhang ST, Yang HJ et al (2011) Gene expression profile analysis of tobacco leaf trichomes. BMC Plant Biol 11:76. https://doi.org/10.1186/1471-2229-11-76
Cunningham FJ, Goh NS, Demirer GS, Matos JL (2018) Nanoparticle-mediated delivery towards advancing plant genetic engineering. Trends Biotechnol 36:882–897. https://doi.org/10.1016/j.tibtech.2018.03.009
D'hoop BB, Paulo MJ, Kowitwanich K et al (2010) Population structure and linkage disequilibrium unravelled in tetraploid potato. Theor Appl Genet 121:1151–1170
Dangl JL, Jones JDG (2001) Plant pathogens and integrated defense responses to infection. Nature 411:826–833

Daniell H, Lin CS, Yu M et al (2016) Chloroplast genomes: diversity, evolution, and applications in genetic engineering. Genome Biol 17:134. https://doi.org/10.1186/s13059-016-1004-2
Darling AC, Mau B, Blattner FR et al (2004) Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14(7):1394–1403. https://doi.org/10.1101/gr.2289704
Darlington CD, Janaki Ammal EK (1945) Chromosome Atlas of cultivated plants. George Allen and Unwin Ltd., London
Darvishzadeh R, Alvi SR, Sarrafi A (2011) Genetic variability for chlorine concentration in oriental tobacco genotypes. Arch Agron Soil Sci 57(2):167–177
Davalieva K, Maleva I, Filiposki K et al (2010) Genetic variability of Macedonian tobacco varieties determined by microsatellite marker analysis. Diversity 2(4):439–449. https://doi.org/10.3390/d2040439
Davarpanah SJ, Jung SH, Kim YJ et al (2009) Stable plastid transformation in *Nicotiana benthamiana*. J Plant Biol 52(3):244–250. https://doi.org/10.1007/s12374-009-9023-0
de Vetten N, Wolters AM, Raemakers K et al (2003) A transformation method for obtaining marker free plants of a cross-pollinating and vegetatively propagated crop. Nat Biotechnol 21:439–442
Del Piano L, Abet M, Sorrentino C et al (2000) Genetic variability in *Nicotiana tabacum* and *Nicotiana* species as revealed by RAPD markers: 1. Development of the RAPD procedure. Beitr Tab Int/contrib Tob Res 19:1–15
Demirer GS, Zhang H, Matos JL et al (2019) High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. Nat Nanotechnol 14:456–464. https://doi.org/10.1038/s41565-019-0382-5
Demirer GS, Silva TN, Jackson CT et al (2021) Nanotechnology to advance CRISPR-Cas genetic engineering of plants. Nat Nanotechnol 12:1–8
Denduangboripant J, Piteekan T, Nantharat M (2010) Genetic polymorphism between tobacco cultivar-groups revealed by amplified fragment length polymorphism analysis. J Agri Sci 2(2):41–48
Dias de Oliveira E, Bramley H, Siddique KH et al (2013) Can elevated CO_2 combined with high temperature ameliorate the effect of terminal drought in wheat? Funct Plant Biol 40:160–171. https://doi.org/10.1071/FP12206
Djanaguiraman M, Prasad PVV (2010) Ethylene production under high temperature stress cause premature leaf senescence in soybean. Funct Plant Biol 37:1071–1084
Doganlar S, Frary A, Daunay MC et al (2002) A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. Genetics 161:1697–1711
East EM (1932) Studies of self-sterility. IX. The behavior of crosses between self-sterile and self-fertile plants. Genetics 17:175–202
Edwards KD, Bombarely A, Story GW et al (2010) Allen F, Mueller LA, Coates SA et al. TobEA: an atlas of tobacco gene expression from seed to senescence. BMC Genomics 11:142
Edwards KD, Fernandez-Pozo N, Drake-Stowe K et al (2017) A reference genome for *Nicotiana tabacum* enables map-based cloning of homeologous loci implicated in nitrogen utilization efficiency. BMC Genomics 18:448. https://doi.org/10.1186/s12864-017-3791-6
EFSA (2012) Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. EFSA J 10(2):2561
El-Morsy SHI, Dorra MDM, Abd El-Hady EAA (2009) Comparative studies on diploid and tetraploid levels of *Nicotiana alata.* Acad J Plant Sci 2(3):182–188
Esau K (1960) Anatomy of seed plants. Soil Sci 90:149
European Commission (1989) Genetic transformation of plant mitochondria: development of a general strategy. https://cordis.europa.eu/project/id/BAP*0102. Accessed 21 Sept 2021
Evanno G, Reganut E, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620
Fan W, Sun X, Yang A (2015) Exploring high-potassium favorable allele mutation of tobacco based on genome-wide association analysis. Acta Tab Sin 25:11–19

FAO (2009) International treaty on plant genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome
FAO (2017) The international treaty on plant genetic resources for food and agriculture: farmers' rights. Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/3/I7820EN/i7820en.pdf
FAO (2019) FAOSTAT statistical database. Food and Agriculture Organization of the United Nations. Rome. http://www.fao.org/faostat/en/#data/QCL
Fita A, Rodríguez-Burruezo A, Boscaiu M et al (2015) Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. Front Plant Sci 6:978. https://doi.org/10.3389/fpls.2015.00978
Flint-Garcia SA, Thornsberry JM, Buckler ES (2003) Structure of linkage disequilibrium in plants. Annu Rev Plant Biol 54:357–374. https://doi.org/10.1146/annurev.arplant.54.031902.134907
Flowers T (2004) Improving crop salt tolerance. J Exp Bot 55:307–319
Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol 179:945–963. https://doi.org/10.1111/j.1469-8137.2008.02531.x
Fontes RLS, Cox FR (1998) Zinc toxicity in soybean grown at high iron concentration in nutrient solution. J Plant Nutr 21:1723–1730
Franco-Navarro JD, Brumós J, Rosales MA et al (2016) Chloride regulates leaf cell size and water relations in tobacco plants. J Exp Bot 67:873–891
Franco-Navarro JD, Rosales MA, Cubero-Font P et al (2019) Chloride as macronutrient increases water use efficiency by anatomically-driven reduced stomatal conductance and increased mesophyll diffusion to CO_2. Plant J 99:1–17
Fricano A, Bakaher N, Corvo MD et al (2012) Molecular diversity, population structure, and linkage disequilibrium in a worldwide collection of tobacco (*Nicotiana tabacum* L.) germplasm. BMC Genetics 13:18. https://doi.org/10.1186/1471-2156-13-18
Friebe B, Jiang J, Raupp WJ et al (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59–87
Fu YQ, Li LH, Wang PW et al (2012) Delivering DNA into plant cell by gene carriers of ZnS nanoparticles. Chem Res Chin Univ 28:672–676
Fuentes P, Armarego-Marriott T, Bock R (2018) Plastid transformation and its application in metabolic engineering. Curr Opin Biotechnol 49:10–15. https://doi.org/10.1016/j.copbio.2017.07.004
Ganal MW, Altmann T, Roder MS (2009) SNP identification in crop plants. Curr Opin Plant Biol 12:211–217
Ganesh CT, Saiprasad GVS, Mohan Raju B et al (2014) Genetic structure in FCV tobacco population as assessed by multi-locus genotyping using SSR markers. Adv Crop Sci Technol 2:3. https://doi.org/10.4172/2329-8863.1000127
Gangwar S, Singh VP (2011) Indole acetic acid differently changes growth and nitrogen metabolism in Pisum sativum L seedlings under chromium (VI) phytotoxicity: implication of oxidative stress. Sci Hort 129:321–328. https://doi.org/10.1016/j.scienta.2011.03.026
Gaskell G, Allansdottir A, Allum N et al (2011) The 2010 Eurobarometer on the life sciences. Nat Biotechnol 29:113–114
Gholizadeh S, Darvishzadeh R, Mandoulakani BA et al (2012) Molecular characterization and similarity relationships among flue-cured tobacco (*Nicotiana tabacum* L.) genotypes using simple sequence repeat markers. Not Bot Hort Agrobot 40(1):247–253
Gier LJ (1940) Root systems of bright belt tobacco. Amer J Bot 27(9):780–787
GM approval database (2021) International Service for the Acquisition of Agri-biotech Applications (ISAAA), New York. GM Crops List—GM Approval DatabaseISAAA.org. Accessed 21 Sept 2021
Goggin FL, Lorence A, Topp CN (2015) Applying high-throughput phenotyping to plant–insect interactions: picturing more resistant crops. Curr Opin Insect Sci 9:69–76. https://doi.org/10.1016/j.cois.2015.03.002

Golestanipour A, Nikkhah M, Aalami A et al (2018) Gene delivery to tobacco root cells with single-walled carbon nanotubes and cell-penetrating fusogenic peptides. Mol Biotechnol 60:863–878. https://doi.org/10.1007/s12033-018-0120-5

Gong D, Huang L, Xu X et al (2016) Construction of a high-density SNP genetic map in flue-cured tobacco based on SLAF-seq. Mol Breed 36:100

Goodspeed TH (1954) The genus *Nicotiana*: origins, relationships and evolution of its species in the light of their distribution, morphology and cytogenetics. Chronica Botanica, Waltham, Mass, USA

Goodspeed TH, Thompson MC (1959) Cytotaxonomy of *Nicotiana* II. Bot Rev 25:385–415

Gopalachari NC (1984) Tobacco. Indian Council of Agricultural Research, New Delhi

Grattan S (2002) Irrigation water salinity and crop production. UCANR Publications, Oakland, CA. https://doi.org/10.1007/s00425-003-1105-5

Greenberg S (2018) A review of participatory plant breeding and lessons for African seed and food sovereignty movements. Published by The African Centre for Biodiversity, Johannesburg, South Africa. https://www.researchgate.net/publication/323771637

Grotewold E (2008) Transcription factors for predictive plant metabolic engineering: are we there yet? Curr Opin Biotechnol 19(2):138–144. https://doi.org/10.1016/j.copbio.2008.02.002

Guardiola JM, Perez O, Diaz L (1987) Effect of chlorine and potassium on combustibility from fine plantations. Tabacco 10:29–43

Guha A, Rasineni GK, Reddy AR (2010) Drought tolerance in mulberry (*Morus* spp.) a physiological approach with insight in to growth dynamics and leaf yield production. Exp Agri 46:471–488

Guo JR, Li YD, Han GL et al (2018) NaCl markedly improved the reproductive capacity of the euhalophyte Suaeda salsa. Funct Plant Biol 45:350–361. https://doi.org/10.1071/fp17181

Guo JR, Suo SS, Wang BS (2015) Sodium chloride improves seed vigour of the euhalophyte Suaeda salsa. Seed Sci Res 25:335–344. https://doi.org/10.1017/s0960258515000239

Guo YH, Jia WJ, Song J et al (2012) *Thellungilla halophila* is more adaptive to salinity than *Arabidopsis thaliana* at stages of seed germination and seedling establishment. Acta Physiol Plant 34:1287–1294

Gyula P, Schäfer E, Nagy F (2003) Light perception and signalling in higher plants. Curr Opin Plant Biol 6:446–452

Harada E, Kim J, Meyer et al (2010) Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. Plant Cell Physiol 51(10):1627–1637

Harborne JB (1986) The flavonoids. Advances in research since 1986. Chapmand Hall, London

Hardon J, Norbu U, Nguyen S et al (2005) Evaluation report of the Biodiversity Use and Conservation in Asia Programme (BUCAP). NORAD, Oslo

Hatami MH, Karimzadeh G, Darvishzadeh R et al (2013) Identification of QTLs associated with low chloride accumulation in oriental tobacco. Genetika 45:55–864

Havlov M, Dobrev PI, Motyka V et al (2008) The role of cytokinins in responses to water deficit in tobacco plants over-expressing *trans*-zeatin O-glucosyltransferase gene under *35S* or *SAG12* promoters. Plant Cell Environ 31:341–353

He M, He C-Q, Ding N-Z (2018) Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. Front Plant Sci 9:1771

Hefferon KL (2010) Plant biotechnology patents: applications in agriculture and medicine. Recent Pat Biotechnol 4. https://doi.org/10.2174/187220810791110723

Heggestad HE (1966) Ozone as a tobacco toxicant. J Air Poll Contr Assoc 16:691–694

Heggestad HE, Middleton JT (1959) Ozone in high concentration as cause of tobacco leaf injury. Science 129:208–210

Higase S (1959) Physiological studies on the flower formation of tobacco: effects of water-logging after transplanting stage on the developmental responses of tobacco. Jpn J Crop Sci 27(2):299–300

Hirohata A, Sato I, Kaino K et al (2019) CRISPR/Cas9-mediated homologous recombination in tobacco. Plant Cell Rep 38:463–473

Holme IB, Wendt T, Holm PB (2013) Intragenesis and cisgenesis as alternatives to transgenic crop development. Plant Biotechnol J 11(4):395–407. https://doi.org/10.1111/pbi.12055

Honig A, Marton I, Rosenthal M et al (2015) Transient expression of virally delivered meganuclease in planta generates inherited genomic deletions. Mol Plant 8(8):1292–1294. https://doi.org/10.1016/j.molp.2015.04.001

Hospital F (2003) Marker-assisted breeding. In: Newbury HJ (ed) Plant molecular breeding. Blackwell Publishing and CRC Press, Oxford and Boca Raton, pp 30–59. https://doi.org/10.1007/978-3-642-21201-7_10

Hou H, Atlihan N, Lu Z-X (2014) New biotechnology enhances the application of cisgenesis in plant breeding. Front Plant Sci 5:389. https://doi.org/10.3389/fpls.2014.00389

Huang W, Yang YJ, Hu H (2016) Response of the water–water cycle to the change in photorespiration in tobacco. J Photochem Photobiol B 157:97–104

Huang WK, Guo JY, Wan FH et al (2008) AFLP analyses on genetic diversity and structure of *Eupatorium adenophorum* populations in China. Chin J Agri Biotechnol 5:33–41

Hurd EA (1971) Can we breed for drought resistance? In: Larson LL, Eastin JD (eds) Drought injury and resistance in crops. Crop Sci Soc America USA, pp 77–78

ICAR (2015) Vision 2050: Central Tobacco Research Institute. Indian Council of Agricultural Research, New Delhi

ICAR-CTRI (2016) Annual report 2015–16. ICAR-Central Tobacco Research Institute, Rajahmundry, AP, India

ICAR-CTRI (2021) List of released/identified varieties of various tobacco types for cultivation in India. https://ctri.icar.gov.in/files/varieties.pdf. Accessed 25 Feb 2021

IPCC (2019) Summary for policymakers. In: Shukla PR, Skea J, Calvo Buendia E et al (eds) Climate change and land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. World Meteorological Organization, Geneva, Switzerland (in press)

ISAAA (2019) Global status of commercialized biotech/GM crops. ISAAA Brief 55–2019. https://www.isaaa.org/resources/publications/briefs/55/default.asp9. Accessed 30 Sept 2021

ISAAA (2020) Pocket K No. 42 stacked traits in biotech crops. https://www.isaaa.org/resources/publications/pocketk/42/default.asp. Accessed 30 Sept 2021

Jacobs JME, Van Eck HJ, Arens P et al (2004) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. Theor Appl Genet 91:289–300

Jafar M, Aggarwal R, Ramesh B et al (2012) SNP markers and their impact on plant breeding. Int J Plant Genom 2012. https://doi.org/10.1155/2012/728398

Janardhan KV, Nataraju SP, Gurumurthy BR et al (1994) Screening flue-cured tobacco genotypes to drought using line source technique. Tob Res 20(1):16–20

Jarup L, Berglund M, Elinder CG et al (1998) Health effects of cadmium exposure—a review of the literature and a risk estimate. Scand J Work Environ Health 24:1–51

Jarvis A, Lane A, Hijmans RJ (2008) The effect of climate change on crop wild relatives. Agri Ecosyst Environ 126:13–23

Jenkins GI (2014) The UV-B photoreceptor UVR8: from structure to physiology. Plant Cell 26:21–37

Jiang G-L (2013) Molecular markers and marker-assisted breeding in plants. In: Andersen SB (ed) Plant breeding from laboratories to fields. IntechOpen. https://doi.org/10.5772/52583

Jingjing JIN (2018) Tobacco genomic resource. https://www.coresta.org/abstracts/tobacco-genomic-resource-31854.html. Accessed 30 Sept 2021

Jinks JL, Jayasekara NEM, Boughey H (1977) Joint selection for both extremes of mean performance and of sensitivity to a macro—environmental variable. II Single seed descent. Heredity 39:345–355

Johnson GR, Frey KJ (1967) Heritability of quantitative attributes of Oats at varying levels of environmental stress. Crop Sci 7:43–46

Johnston S, Anziano P, Shark K et al (1988) Mitochondrial transformation in yeast by bombardment with microprojectiles. Science 240:1538–1541

Jones KJ, Shew HD (1995) Early season root production and zoospore infection of cultivars of flue-cured tobacco that differ in level of partial resistance to Phytophthora parasitica var. nicotianae. Plant Soil 172(1):55–61

Juan CM, Mi J, Shreya A, Kössler S et al (2020) Expression of a carotenogenic gene allows faster biomass production by redesigning plant architecture and improving photosynthetic efficiency in tobacco. Plant J Cell Mol Biol. https://doi.org/10.1111/tpj.14909

Juan R, del CN (1986) Irrigation water management and chemical and physical characteristics of covered dark tobacco. Riego y Drenaje 9:71–83

Jube S, Borthakur D (2007) Expression of bacterial genes in transgenic tobacco: methods, applications and future prospects. Elec J Biotechnol 10(3):452–467. https://doi.org/10.2225/vol10-issue3-fulltext-4

Julio E, Denoyes-Rothan B, Verrier JL et al (2006) Detection of QTLs linked to leaf and smoke properties in *Nicotiana tabacum* based on a study of 114 recombinant inbred lines. Mol Breed 18:69–91

Jung J, Kim JI, Yi G (2019) geneCo: a visualized comparative genomic method to analyze multiple genome structures. Bioinformatics 35(24):5303–5305. https://doi.org/10.1093/bioinformatics/btz596

Kaličanin B, Velimirović D (2012) Potentiometric stripping analysis of zinc cadmium and lead in tobacco leaves (*Nicotiana Tabacum* L) and soil samples. Intl J Electrochem Sci 7:313–323

Kamalay JC, Goldberg RB (1980) Regulation of structural gene expression in tobacco. Cell 19:935–946. https://doi.org/10.1016/0092-8674(80)90085-9

Kami C, Lorrain S, Hornitschek P et al (2010) Light-regulated plant growth and development. Curr Topics Dev Biol 91:29–66

Karaivazoglou NA, Papakosta DK, Divanidis S (2005) Effect of chloride in irrigation water and form of nitrogen fertilizer on Virginia (flue cured) tobacco. Field Crops Res 92:61–74

Karaivazoglou NA, Tsotsolis NC, Tsadilas CD (2007) Influence of liming and form of Sci 100 nitrogen fertilizer on nutrient uptake growth yield and quality of Virginia (flue-cured) tobacco. Field Crops 100:52–60

Kasuga M, Miura S, Shinozaki K et al (2004) Combination of the Arabidopsis DREB1A gene and stress-inducible rd29A promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. Plant Cell Physiol 45:346–350

Kenton A, Parokonny AS, Gleba YY et al (1993) Characterization of the *Nicotiana tabacum* L. genome by molecular cytogenetics. Mol Gen Genet 240:159–169

Khan SH (2019) Genome-editing technologies: concept, pros and cons of various genome-editing techniques and bioethical concerns for clinical application. Mol Ther Nucleic Acids 16:326–334. https://doi.org/10.1016/j.omtn.2019.02.027

Kim JW, Shon JY, Lee CK et al (2011) Relationship between grain filling and leaf senescence of temperate rice under high temperature. Field Crop Res 122:207–213

King MJ (1990) Tobacco. In: Stewart BA, Nielsen DR. Irrigation of agricultural crops. Agronomy Series, vol 30. American Society of Agronomy Inc, Madison (WI), pp 811–833

Knapp S, Chase MW, Clarkson JJ (2004) Nomenclatural changes and a new sectional classification in *Nicotiana* (Solanaceae). Taxon 52:73–82

Kole C (2017) Aims and scope of the volume, genomic designing of climate smart cereal crops. Proposal submitted to Springer Nature.

Kolodyazhnaya YS, Kutsokon NK, Levenko BA et al (2009) Transgenic plants tolerant to abiotic stresses. Cytol Genet 43:132–149

Komarnitsky SI, Komarnitsky IK, Cox A et al (1998) Molecular phylogeny of the nuclear 5.8S ribosomal RNA genes in 37 species of *Nicotiana* genus. Genetika 34:883–889

Kostoff D (1943) An androgenic *Nicotiana* haploid. Zeits. F Zelloysch U mikr Anat 9:640–642

Kramer PJ (1951) Causes of injury to plants resulting from flooding of the soil. Plant Physiol 26:722–736

Kramer PJ, Jackson WT (1954) Causes of injury to flooded tobacco plants. Plant Physiol 2(3):241–245

Kuchuk N, Sytnyk K, Vasylenko M et al (2006) Genetic transformation of plastids of different Solanaceae species using tobacco cells as organelle hosts. Theor Appl Genet 113:519–527. https://doi.org/10.1007/s00122-006-0318-y

Kwak SY, Lew TTS, Sweeney CJ et al (2019) Chloroplast-selective gene delivery and expression in planta using chitosan-complexed single-walled carbon nanotube carriers. Nat Nanotechnol 14:447–455. https://doi.org/10.1038/s41565-019-0375-4

Lambers H, Chapin FS III, Pons TL (2008) Plant physiological ecology, 2nd edn. Springer, New York

Lander ES, Green P, Abrahamson J et al (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1(2):174–181. https://doi.org/10.1016/0888-7543(87)90010-3

Lecours N, Almeida GEG, Abdallah JM et al (2012) Environmental health impacts of tobacco farming: a review of the literature. Tob Control 21:191–196

Legg PD, Litton CC, Collins GB (1981) Effects of the *Nicotiana debneyi* black root rot resistance factor on agronomic and chemical traits in burley tobacco. Theor Appl Genet 60:365–368

Legg PD, Smeeton BW (1999) Breeding and genetics. In: David DL, Nielsen MT (eds) Tobacco production, chemistry and technology. Blackwell Science, Malden, MA, USA

Leitch IJ, Hanson L, Lim KY et al (2008) The ups and downs of genome size evolution in polyploid species of *Nicotiana* (Solanaceae). Ann Bot 101:805–814

Lester RN, Hawkes JG (2001) Solanaceae. In: Hanelt P (ed) Mansfeld's encyclopedia of agricultural and horticultural crops (except ornamentals), vol 4. Springer Berlin

Levitt J (1972) Responses on plants to environmental stresses. Academic Press, New York, NY

Lewis RS (2005) Transfer of resistance to potato virus Y (PVY) from *Nicotiana africana* to *Nicotiana tabacum*: possible influence of tissue culture on the rate of introgression. Theor Appl Genet 110:678–687

Lewis RS (2011) Nicotiana. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, plantation and ornamental crops. Springer, Berlin, Heidelberg

Li S, Chang L, Zhang J (2021) Advancing organelle genome transformation and editing for crop improvement. Plant Commun 2(2):100141. https://doi.org/10.1016/j.xplc.2021.100141

Li XH, Xing XX, Xu SX et al (2018) Genome-wide identification and functional prediction of tobacco lncRNAs responsive to root-knot nematode stress. PLoS One 13(11):15

Lidon FJC (2012) Micronutrients' accumulation in rice after supplemental UV-B irradiation. J Plant Interact 7:19–28

Liharska TB, Koornneef M, van Wordragen M et al (1996) Tomato chromosome 6: effect of alien chromosomal segments on recombinant frequencies. Genome 39:485–491

Li-Hua L, Mei-Xia C, Dong-Xin Z et al (2011) QTL analysis of six important traits in tobacco (*Nicotiana tabacum* L.). Acta Agron Sin 37:1577–1584

Lim KY, Kovarik A, Matyasek R et al (2007) Sequence of events leading to near complete genome turnover in allopolyploid Nicotiana within five million years. New Phytol 175:756–763

Lim KY, Matyasek R, Kovarik A et al (2004) Genome evolution in allotetraploid *Nicotiana*. Biol Linn Soc 82:599–606

Lim KY, Matyasek R, Lichtenstein CP, Leitch AR et al (2000) Molecular cytogenetic analyses and phylogenetic studies in the *Nicotiana* section Tomentosae. Chromosoma 109:245–258

Lim KY, Matyasek R, Kovarik A et al (2005) Molecular cytogenetics and tandem repeat sequence evolution in the allopolyploid *Nicotiana rustica* compared with diploid progenitors *N. paniculata* and *N. undulate*. Cytogenet Genome Res 109:298–309. https://doi.org/10.1159/000082413

Lin TY, Kao YY, Lin S et al (2001) A genetic linkage map of *Nicotiana plumbaginifolia*/*Nicotiana longiflora* based on RFLP and RAPD markers. Theor Appl Genet 103:905–911

Liu LH, Shao ZY, Zhang M et al (2015) Regulation of carotenoid metabolism in tomato. Mol Plant 8:28–39

Liu XZ, He CS, Yang YM et al (2009) Genetic diversity among flue-cured tobacco cultivars on the basis of AFLP markers. Czech J Genet Plant Breed 45(4):155–159

Liu XZ, Zhang HY (2008) Advance of molecular marker application in the tobacco research. Afr J Biotechnol 7(25):4827–4831
Livingstone KD, Lackney VK, Blauth JR et al (1999) Genome mapping in Capsicum and the evolution of genome structure in the Solanaceae. Genetics 152:1183–1202
Long SP, Marshall-Colon A, Zhu XG (2015) Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell 161(1):56–66. https://doi.org/10.1016/j.cell.2015.03.019
Lu JP (1997) The application of PAGE in the cultivars identification of flue-cured tobacco. Seed 5:30–32
Lu XP, Gui YJ, Xiao BG et al (2012) Development of DArT markers for a linkage map of flue-cured tobacco. Chin Sci Bull 58:641–648
Lu Y, Stegemann S, Agrawal S et al (2017) Horizontal transfer of a synthetic metabolic pathway between plant species. Curr Biol 19:3034–3041
Lu YH, Rijzaani H, Karcher D et al (2013) Efficient metabolic pathway engineering in transgenic tobacco and tomato plastids with synthetic multigene operons. Proc Natl Acad Sci USA E623–E632
Lücker J, Schwab W, Hautum B et al (2004) Increased and altered fragrance of tobacco plants after metabolic engineering using three monoterpene synthases from lemon. Plant Physiol 134:510–519. https://doi.org/10.1104/pp.103.030189
Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. In: Brady NC (ed) Advances in agronomy, vol 43. Academic Press, pp 107–153
Lugon-Moulin N, Martin F, Krauss MR et al (2008) Arsenic concentration in tobacco leaves: a study on three commercially important tobacco (*Nicotiana tabacum* L) types. Water Air Soil Pollut 192:315–319
Lugon-Moulin N, Ryan L, Donini P et al (2006) Cadmium content of phosphate fertilizers used for tobacco production. Agron Sustain Dev 26:151–155
Ma HB, Qi JM, Li YK et al (2008) Construction of a molecular genetic linkage map of tobacco based on SRAP and ISSR markers. Acta Agron Sin 34:1958–1963
Macková H, Hronková M, Dobrá J et al (2013) Enhanced drought and heat stress tolerance of tobacco plants with ectopically enhanced cytokinin oxidase/dehydrogenase gene expression. J Exp Bot 64(10):2805–2815. https://doi.org/10.1093/jxb/ert131
Madhav MS, Raju KS, Gaikwad K et al (2015) Development of new set of microsatellite markers in cultivated tobacco and their transferability in other *Nicotiana* spp. Mol Plant Breed 6(16):1–13
Maliga P, Svab Z (2010) Engineering the plastid genome of *Nicotiana sylvestris*, a diploid model species for plastid genetics. In: Birchler J (ed) Plant chromosome engineering: methods and protocols. Humana Press, Totowa. https://doi.org/10.1007/978-1-61737-957-4_2
Manly KF, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 12(12):930–932. https://doi.org/10.1007/s00335-001-1016-3
Matsuoka K, Demura T, Galis I et al (2004) A comprehensive gene expression analysis toward the understanding of growth and differentiation of tobacco BY-2 cells. Plant Cell Physiol 45(9):1280–1289. https://doi.org/10.1093/pcp/pch155
Matyasek R, Lim KY, Kovarik A et al (2003) Ribosomal DNA evolution and gene conversion in *Nicotiana rustica*. Heredity (Edinb) 91(3):268–275. https://doi.org/10.1038/sj.hdy.6800333
McEvoy ET (1957) The growth and mineral content of flue-cured tobacco as influenced by reaction of nutrient solutions with ionic forms of nitrogen. Can J Soil Sci 37:79–83
McKee CG (1968) Maryland 64, a high yielding, later maturing variety of Maryland tobacco. Md Agrl Exp Sta Misc Publ 654:4
Meharg AA, Macnair MR (1992) Suppression of the high affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L. J Exp Bot 43:519–524
Mehmood F, Abdullah, Ubaid Z et al (2020) Plastid genomics of *Nicotiana* (Solanaceae): insights into molecular evolution, positive selection and the origin of the maternal genome of Aztec tobacco (*Nicotiana rustica*) Peer J 8:e9552. https://doi.org/10.7717/peerj.9552

Meissner R, Chague V, Zhu Q et al (2000) Technical advance: a high throughput system for transposon tagging and promoter trapping in tomato. Plant J 22:265–274. https://doi.org/10.1046/j.1365-313x.2000.00735.x

Menser HA, Street OE (1962) Effects of air pollution, nitrogen levels, supplemental irrigation, and plant spacing on weather fleck and leaf losses of Maryland tobacco. Tob Sci 6:165–169

Mielby H (2011) Public attitudes to Cisgenic crops. PhD thesis, University of Copenhagen

Milla SR, Levin JS, Lewis RS et al (2005) RAPD and SCAR markers linked to an introgressed gene conditioning resistance to *Peronospora tabacina* D.B. Adam. in tobacco. Crop Sci 45:2346–2354

Miransari M (2011) Hyperaccumulators arbuscular mycorrhizal fungi and stress of heavy metal. Biotechnol Adv 29:645–653. https://doi.org/10.1016/jbiotechadv201104006

Moon HS, Nicholson JS, Heineman A et al (2009a) Changes in genetic diversity of U.S. flue-cured tobacco germplasm over seven decades of cultivar development. Crop Sci 49(2):498–508. https://doi.org/10.2135/cropsci2008.05.0253

Moon HS, Nicholson JS, Lewis RS (2008) Use of transferable *Nicotiana tabacum* L. microsatellite markers for investigating genetic diversity in the genus *Nicotiana.* Genome 51:547–559

Moon HS, Nifong JM, Nicholson JS et al (2009b) Microsatellite-based analysis of tobacco (*Nicotiana tabacum* L.) genetic resources. Crop Sci 49:1–11. https://doi.org/10.2135/cropsci2009.01.0024

Morgan JM (1984) Osmoregulation and water stress in higher plants. Annu Rev Plant Physiol 35:299–319

Morris M, Dreher K, Ribaut JM (2003) Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. Mol Breed 11:235–247. https://doi.org/10.1023/A:1022872604743

Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167(3):645–663

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

Murad L, Lim KY, Christopodulou V et al (2002) The origin of tobacco's T genome is traced to a particular lineage within *Nicotiana tomentosiformis* (Solanaceae). Amer J Bot 89:921–928

Muttaqin AS, Suarma U, Nurjani E et al (2019) The impact of climate variability on tobacco productivity over Temanggung Regency, Indonesia. E3S Web Confer 76:04003. https://doi.org/10.1051/e3sconf/20197604003. ICST 2018

Myles S, Peiffer J, Brown PJ et al (2009) Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell 21:2194–2202. https://doi.org/10.1105/tpc.109.068437

Narayan RK (1987) Nuclear DNA changes, genome differentiation and evolution in *Nicotiana* (Solanaceae). Plant Syst Evol 157(04):161–180

Narayanan AI, Murthy TGK, Sarala K et al (2003) Division of crop improvement. Information bulletin. Central Tobacco Research Institute, Rajahmundry

Nascimento NC, Fett-Neto AG (2010) Plant secondary metabolism and challenges in modifying its operation: an overview. In: Fett-Neto A (ed) Plant secondary metabolism engineering. Methods in molecular biology (methods and protocols), vol 643. Humana Press, Totowa

Navabpour S, Morris K, Allen R et al (2003) Expression of senescence-enhanced genes in response to oxidative stress. J Exp Bot 54:2285–2292

Ndunguru BJ, Ntare BR, Williams JH, Greenberg DC (1995) Assessment of groundnut cultivars for end-of-season drought tolerance in a Sahelian environment. J Agri Sci 125:79–85

Niazian M, Sadatnoori SA, Galuszka P et al (2017) Tissue culture-based Agrobacterium-mediated and in Planta transformation methods. Czech J Genet Plant Breed 53:133–143. https://doi.org/10.17221/177/2016-CJGPB

Nisar N, Li L, Lu S et al (2015) Carotenoid metabolism in plants. Mol Plant 8:68–82

Nishi T, Tajima T, Noguchi S et al (2003) Identification of DNA markers of tobacco linked to bacterial wilt resistance. Theor Appl Genet 106(4):765–770. https://doi.org/10.1007/s00122-002-1096-9

Nishiyama R, Watanabe Y, Fujita Y et al (2011) Tran. LSP Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought,

salt and ABA responses, and ABA biosynthesis. Plant Cell 23:2169–2183. https://doi.org/10.1105/tpc.111.087395
Nobel PS (1980) Leaf anatomy and water use efficiency. In: Turner NC, Kramer PJ (eds) Adaptation of plants to water and high temperature stress. Wiley, New York, pp 43–55
Nordberg G, Nogawa K, Nordberg M et al (2007) Handbook on toxicology of metals. Academic Press, New York
Norom IC, Osibanjo O, Oji-nnorom CG (2005) Cadmium determination in cigarettes available in Nigeria. Afr J Biotechnol 4:1128–1132
Nurhidayati T, Wardhani SP, Purnobasuki H et al (2017) Response morphology and anatomy of tobacco (*Nicotiana tabacum* L.) plant on waterlogging. Conf Proc 1908:040009. https://doi.org/10.1063/1.5012723
Oeung S, Voleak N, Huykhim U et al (2017) Phytochemical analysis of different extracts of leaves of *Nicotiana tabacum* L of Cambodia. Asian J Pharm 1(3):18–26
Okumus A, Gulumser A (2001) Chiasma frequency chromosome pairing at tobacco genotypes in the Middle Black Sea region. Online J Biol Sci 1(7):612–613
Opperman CH, Lommel SA, Sosinski BR et al (2003) The tobacco genome initiative Paper presented at CORESTA Meeting, Agronomy/Phytopathology, Bucharest, Romania, 12–17 Oct 2003
Ort DR, Merchant SS, Alric J et al (2015) Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc Natl Acad Sci USA. https://doi.org/10.1073/pnas.1424031112
Pallardy SG (2010) Physiology of woody plants. Academic Press, London
Passioura JB (1983) Roots and drought resistance. Agri Water Manage 7:265–280
Paterson AH, DeVerna JW, Lanini B et al (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics 124(3):735–742. https://doi.org/10.1093/genetics/124.3.735
Pereira JS, Chaves MM (1993) Plant water deficits in Mediterranean ecosystems. In: Smith JAC, Griffiths H (eds) Plant responses to water deficits–from cell to community. BIOS Scientific Publishers, Oxford, pp 237–251
Perez-Alfocea F, Balibrea ME et al (1996) Agronomical and physiological characterization of salinity tolerance in a commercial tomato hybrid. Plant Soil 180:251–257
Pilon-Smits EAH, Terry N, Sears T et al (1998) Trehalose-producing transgenic tobacco plants show improved growth performance under drought stress. J Plant Physiol 152:525–532
Popov VN, Antipina OV, Burakhanova EA (2013) Involvement of cell-wall invertase in low-temperature hardening of tobacco plants. Russ J Plant Physiol 2:221–226
Povilaitis B, White FH (1966) Delcrest 66, a new variety of bright tobacco. Can J Plant Sci 46:458
Prabhakararao K, Sarala K, Murthy TGK (2016) Patent mapping of disease resistance genes in tobacco. J Agri Crop Sci 3:01–07
Prabhakararao K, Sarala K, Murthy TGK et al (2012) Application of Simple Sequence Repeat (SSR) markers in diversity analysis of tobacco types. Tob Res 38(1):29–33
Pritchard JK, Stephens M, Rosenberg NA et al (2000) Association mapping in structured populations. Am J Hum Genet 67(1):170–181
Puchta H (1999) Use of I-Sce I to induce DNA double-strand breaks in *Nicotiana*. Methods Mol Biol 113:447–451
Purrington CB (2000) Costs of resistance. Curr Opin Plant Biol 3:305–308
Qi JM, Wang T, Chen SH et al (2006) Genetic diversity and genetic relatives analysis of tobacco germplasm based on inter-simple sequence repeat (ISSR). Acta Agron Sin 32:373–378
Rabara RC, Tripathi P, Reese RN et al (2015) Tobacco drought stress responses reveal new targets for Solanaceae crop improvement. BMC Genomics 16:484
Rabara RC, Tripathi P, Rushton PJ (2017) Comparative metabolome profile between tobacco and soybean grown under water-stressed conditions. BioMed Res Intl. https://doi.org/10.1155/2017/3065251
Rabbani MA, Maruyama K, Aba H et al (2003) Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. Plant Physiol 133:1755–1767

Rafalski JA (2010) Association genetics in crop improvement. Curr Opin Plant Biol 13:174–180. https://doi.org/10.1016/j.pbi.2009.12.004

Rascon-Cruz Q, Gonzalez-Barriga CD, Blanca Flor Iglesias-Figueroa BF et al (2021) Plastid transformation: advances and challenges for its implementation in agricultural crops. Electron J Biotechnol 51:95–109. https://doi.org/10.1016/j.ejbt.2021.03.005

Rastas P (2017) Lep-MAP3: robust linkage mapping even for low-coverage whole genome sequencing data. Bioinformatics 33(23):3726–3732. https://doi.org/10.1093/bioinformatics/btx494

Rathinasabapathi B (2000) Metabolic engineering for stress tolerance: installing osmoprotectant synthesis pathways. Ann Bot 86:709–716

Reddy AM, Kumar SG, Jyonthsnakumari G et al (2005) Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam) Verdc) and bengalgram (*Cicer arietinum* L). Chemosphere 60:97–104

Regassa G, Chandravanshi B (2016) Levels of heavy metals in the raw and processed Ethiopian tobacco leaves. Springer Plus 5:232

Remacle C, Cardol P, Coosemans N et al (2006) High-efficiency biolistic transformation of Chlamydomonas mitochondria can be used to insert mutations in complex I genes. Proc Natl Acad Sci USA 103:4771–4776

Ren M, Zhang C, Jiang C (2014) Association analysis of tobacco aroma constituents based on high density SSR linkage group. Acta Tab Sin 20:88–93

Ren N, Timko MP (2001) AFLP analysis of genetic polymorphism and evolutionary relationships among cultivated and wild *Nicotiana* species. Genome 44:559–571

Renny-Byfield S, Michael C, Ales et al (2011) Next generation sequencing reveals genome downsizing in allotetraploid *Nicotiana tabacum*, predominantly through the elimination of paternally derived repetitive DNAs. Mol Biol Evol 28:2843–2854

Rhodes D, Samaras Y (1994) Genetic control of osmoregulation in plants. In: Strange K (ed) Cellular and molecular physiology. CRC Press, Boca Raton, pp 347–367

Ribaut J-M, Hoisington D (1998) Marker-assisted selection: new tools and strategies. Trends Plant Sci 3:236–239. https://doi.org/10.1016/S1360-1385(98)01240-0

Rivero RM, Shulaev V, Blumwald E (2009) Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit. Plant Physiol 150:1530–1540. https://doi.org/10.1104/pp.109.139378

Robertson MJT, Fukai S, Peoples MB (2004) The effect of timing and severity of water deficit on growth, development, yield accumulation and nitrogen fixation of mung bean. Field Crops Res 86:67–80

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol 57:675–709

Rommens CM, Humara JM, Ye J et al (2004) Crop improvement through modification of the plant's own genome. Plant Physiol 135:421–431. https://doi.org/10.1104/pp.104.040949

Rontein D, Basset G, Hanson AD (2002) Metabolic engineering of osmoprotectant accumulation in plants. Metab Eng 4:49–56

Rossi L, Bindler G, Pijnenburg H et al (2001) Potential of molecular marker analysis for variety identification in processed tobacco. Plant Var Seeds 14:89–101

Rostoks N, Borevitz J, Hedley P et al (2005) Single-feature polymorphism discovery in the barley transcriptome. Genome Biol 6:R54. https://doi.org/10.1186/gb-2005-6-6-r54

Rushton PJ, Bokowiec MT, Han S et al (2008) Tobacco transcription factors: novel insights into transcriptional regulation in the Solanaceae. Plant Physiol 147(1):280–295

Russell AW, Sparrow R (2008) The case for regulating intragenic GMOs. J Agri Environ Ethic 21:153–181. https://doi.org/10.1007/s10806-007-9074-5

Sarala K (2019) Next gen breeding strategies for enhancing FCV Tobacco productivity and quality. Paper presented at national symposium on approaches and strategies for augmenting tobacco farmers' income, Indian Society of Tobacco Sciences, Rajahmundry, 19–20 July 2019

Sarala K, Baghyalakshmi K, Prabhakara Rao K et al (2018) Genetic diversity among mutant germplasm accessions of *Nicotiana tabacum* as determined by morphological parameters. Tob Res 44(2):47–53

Sarala K, Murthy TGK, Krishnamurthy V (2012) Tobacco (*Nicotiana tabacum* L.). In: Bharadwaj DN (ed) Breeding of field crops. Agrobios (India), Jodhpur, India

Sarala K, Nanda C, Baghyalakshmi K et al (2021) Designing tobacco genomes for resistance to biotic stresses. In: Kole C (ed) Genomic designing for biotic stress resistant crops. Springer International Publishing, Switzerland (in press)

Sarala K, Prabhakara Rao K, Murthy TGK et al (2016) Yield gains in Indian flue-cured virginia tobacco: a case study. Int J Agric Sci 8(52):2538–2541

Sarala K, Rao RVS (2008) Genetic diversity in Indian FCV and burley tobacco cultivars. J Genet 87:159–163

Sarala K, Reddy DD, Rao KP (2020) FCJ 11: a high yielding FCV tobacco cultivar for Northern light soils of Andhra Pradesh. Central Tobacco Research Institute, Rajahmundry. Doc No: D1/Pub/FL2

Sarala K, Swamy AVSR, Nageswara Rao K et al (1998) Identifying drought tolerant FCV tobacco genotypes. Paper presented at the national symposium on Indian Tobacco-Problems and Prospects, ISTS, 20–23 January 1998

Sauter A, Davies WJ, Hartung W (2001) The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. J Exp Bot 52:1991–1997

Schaart JG, Krens FA, Pelgrom KTB et al (2004) Effective production of marker-free transgenic strawberry plants using inducible site-specific recombination and a bifunctional selectable marker gene. Plant Biotechnol J 2:233–240. https://doi.org/10.1111/j.1467-7652.2004.00067.x

Schouten HJ, Krens FA, Jacobsen E (2006) Cisgenic plants are similar to traditionally bred plants: international regulations for genetically modified organisms should be altered to exempt cisgenesis. EMBO Rep 7:750–753

Scoccianti V, Crinelli R, Tirillini B et al (2006) Uptake and toxicity of Cr (Cr3+) in celery seedlings. Chemosphere 64:1695–1703

Seki M, Kamei A, Yamaguchi-Shinozaki K et al (2003) Molecular responses to drought, salinity and frost: common and different paths for plant protection. Curr Opin Biotechnol 14:194–199

Senthil-Kumar M, Hema R, Suryachandra TR et al (2010) Functional characterization of three water deficit stress-induced genes in tobacco and Arabidopsis: an approach based on gene down regulation. Plant Physiol Biochem 48(1):35–44. https://doi.org/10.1016/j.plaphy.2009.09.005

Sharma P, Dubey RS (2005) Lead toxicity in plants. Braz J Plant Physiol 17:35–52

Sharma V, Goel P, Kumar S et al (2019) An apple transcription factor, MdDREB76, confers salt and drought tolerance in transgenic tobacco by activating the expression of stress-responsive genes. Plant Cell Rep 38:221–241

Shen J, Jiang CQ, Yan YF et al (2017) Effect of increased UV-B radiation on carotenoid accumulation and total antioxidant capacity in tobacco (*Nicotiana tabacum* L.) leaves. Genet Mol Res 16(1). https://doi.org/10.4238/gmr16018438. PMID: 28290611

Shilpim M, Narendra T (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158

Shinozaki K, Ohme M, Tanaka M et al (1986) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J 5(9):2043–2049

Shinozaki K, Yamaguchi-Shinozaki K (1996) Molecular responses to drought and cold stress. Curr Opin Biotechnol 7:161–167. https://doi.org/10.1016/S0958-1669(96)80007-3

Sierro N, Battey JN, Ouadi S et al (2013a) Reference genomes and transcriptomes of *Nicotiana sylvestris* and *Nicotiana tomentosiformis*. Genome Biol 4(6):R60. https://doi.org/10.1186/gb-2013-14-6-r60

Sierro N, Battey JND, Bovet L et al (2018) The impact of genome evolution on the allotetraploid *Nicotiana rustica*—an intriguing story of enhanced alkaloid production. BMC Genomics 19(1):855. https://doi.org/10.1186/s12864-018-5241-5

Sierro N, Battey JND, Ouadi S et al (2014) The tobacco genome sequence and its comparison with those of tomato and potato. Nat Commun 5. https://doi.org/10.1038/ncomms4833

Sierro N, Oeveren JV, Eijk MJTV et al (2013b) Whole genome profiling physical map and ancestral annotation of tobacco Hicks Broadleaf. Plant J 75(5):880–889. https://doi.org/10.1111/tpj.12247
Silva AT, Nguyen A, Ye C et al (2010) Conjugated polymer nanoparticles for effective siRNA delivery to tobacco BY-2 protoplasts. BMC Plant Biol 10:291. https://doi.org/10.1186/1471-2229-10-291
Singh S, Parihar P, Singh R et al (2016) Heavy metal tolerance in plants: role of transcriptomics proteomics metabolomics and ionomics. Front Plant Sci. https://doi.org/10.3389/fpls201501143
Sivaraju K, Madhav MS, Sharma RK et al (2008) Molecular diversity in Indian tobacco types as revealed by randomly amplified DNA polymorphism. J Plant Biochem Biotechnol 17:51–56
Smith KM (1937) A textbook of plant virus diseases. P Blakiston's Son & Co Inc., Philadelphia
South PF, Cavanagh AP, Liu HW et al (2019) Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science 363(6422):eaat9077. https://doi.org/10.1126/science.aat9077
Srivastava S, Chaudhry V, Mishra A et al (2012) Gene expression profiling through microarray analysis in *Arabidopsis thaliana* colonized by Pseudomonas putida MTCC5279 a plant growth promoting rhizobacterium. Plant Signal Behav 7:235–245. https://doi.org/10.4161/psb18957
Stam P, Zeven C (1981) The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica 30:227–238
Steward R (2018) Economic impact of disclosure requirements in patent applications for 'genetic resources'-based innovation. International Federation of Pharmaceutical Manufacturers and Associations (IFPMA), Geneva Switzerland and Crop Life International, Brussels, Belgium
Stojanovic D, Nikic D, Lazarevic K (2004) The level of nickel in smoker's blood and urine. Cent Eur J Public Health 12:187–189
Suen DF, Wang CK, Lin RF et al (1997) Assignment of DNA markers to Nicotiana sylvestris chromosomes using monosomic alien addition lines. Theor Appl Genet 94:331–337
Suzuki N, Koussevitzky S, Mittler R et al (2012) ROS and redox signaling in the responses of plants to abiotic stress. Plant Cell Environ 35:259–270
Svab Z, Hajdukiewicz P, Maliga P (1990) Stable transformation of plastids in higher plants. Proc Natl Acad Sci USA 87(21):8526–8530. https://doi.org/10.1073/pnas.87.21.8526
Svab Z, Maliga P (1993) High-frequency plastid transformation in tobacco by selection for a chimeric aadA gene. Proc Natl Acad Sci USA 90(3):913–917. https://doi.org/10.1073/pnas.90.3.913
Swiderska K, Reid H, Song Y et al (2011) The role of traditional knowledge and crop varieties in adaptation to climate change and food security in SW China, Bolivian Andes and coastal Kenya. https://pubs.iied.org/sites/default/files/pdfs/migrate/G03338.pdf. Accessed 30 Sept 2021
Tan SL, Yang YJ, Huang W (2020) Moderate heat stress accelerates photoinhibition of photosystem I under fluctuating light in tobacco young leaves. Photosynth Res 144(3):373–382
Tang C, Asseng S, Diatloff E, Rengel Z (2003) Modelling yield losses of aluminium-resistant and aluminium-sensitive wheat due to subsurface soil acidity: effects of rainfall, liming and nitrogen application. Plant Soil 254:349–360
Tanksley SD, Ganal MW, Prince JP et al (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132(4):1141–1160. https://doi.org/10.1093/genetics/132.4.11410-1160
Tarczynski MC, Jensen RG, Bohnert HJ (1992) Expression of a bacterial mtlD gene in transgenic tobacco leads to production and accumulation of mannitol. Proc Natl Acad Sci USA 89:2600–2604
Tarczynski MC, Jensen RG, Bohnert HJ (1993) Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science 259:508–510
Tavakol E, Pakniyat H (2007) Evaluation of some drought resistance criteria at seedling stage in wheat (*Triticum aestivum* L.) Cultivars. Pak J Biol Sci 10:1113–1117. https://doi.org/10.3923/pjbs.2007.1113.1117
Telem RS, Wani SH, Singh NB et al (2013) Cisgenics—a sustainable approach for crop improvement. Curr Genom 14(7):468–476. https://doi.org/10.2174/13892029113146660013

Terashima I (1992) Anatomy of non-uniform photosynthesis. Photosynth Res 31:195–212. https://doi.org/10.1007/BF00035537
Tezuka T, Tsutomu K, Toshiaki M et al (2010) Seven of eight species in *Nicotiana* section *Suaveolentes* have common factors leading to hybrid lethality in crosses with *Nicotiana tabacum*. Ann Bot 106(2):267–276. https://doi.org/10.1093/aob/mcq114
Tezuka T, Marubashi W (2012) Genes in S and T Subgenomes are responsible for hybrid lethality in interspecific hybrids between *Nicotiana tabacum* and *Nicotiana occidentalis*. PLoS One 7(2). https://doi.org/10.1371/journal.pone.0036204
Thimmegowda GC, Ramadoss SK, Kaikala V et al (2018) Whole genome resequencing of tobacco (*Nicotiana tabacum* L.) genotypes and high-throughput SNP discovery. Mol Breed 38:121
Tian Y, Chen K, Li X et al (2020) Design of high-oleic tobacco (*Nicotiana tabacum* L.) seed oil by CRISPR-Cas9-mediated knockout of NtFAD2–2. BMC Plant Biol 20:233
Tian Y, Liu X, Fan C et al (2021) Enhancement of tobacco (*Nicotiana tabacum* L.) seed lipid content for biodiesel production by CRISPR-Cas9-mediated knockout of NtAn1. Front Plant Sci 11:599474. https://doi.org/10.3389/fpls.2020.599474
Tirani MM, Haghjou MM, Ismaili A (2019) Hydroponic grown tobacco plants respond to zinc oxide nanoparticles and bulk exposures by morphological, physiological and anatomical adjustments. Funct Plant Biol 46:360–375
Toda E, Koiso N, Takebayashi A et al (2019) An efficient DNA- and selectable-marker-free genome-editing system using zygotes in rice. Nat Plants 5:363–368. https://doi.org/10.1038/s41477-019-0386-z
Tong Z, Jiang S, He W et al (2021) Construction of high-density genetic map and QTL mapping in *Nicotiana tabacum* backcrossing BC4F3 population using whole-genome sequencing. Czech J Genet Plant Breed 57:102–112. https://doi.org/10.17221/8/2021-CJGPB
Tong Z, Zhou J, Xiu Z (2020) Construction of a high-density genetic map with whole genome sequencing in *Nicotiana tabacum* L. Genomics 112:2028–2033. https://doi.org/10.1016/j.ygeno.2019.11.015
Tong Z, Xiao B, Jiao F et al (2016) Large-scale development of SSR markers in tobacco and construction of a linkage map in flue-cured tobacco. Breed Sci 66(3):381–390. https://doi.org/10.1270/jsbbs.15129
Tong ZJ, Yang ZM, Chen XJ et al (2012) Large-scale development of microsatellite markers in *Nicotiana tabacum* and construction of a genetic map of flue-cured tobacco. Plant Breed 131:674–680
Torney F, Trewyn BG, Lin VSY et al (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nat Nanotechnol 2:295–300
Townsend JA, Wright DA, Winfrey RJ et al (2009) High-frequency modification of plant genes using engineered zinc-finger nucleases. Nature 459:442–445
Trindade LM, Horvath B, Bachem C et al (2003) Isolation and functional characterization of a stolon specific promoter from potato (*Solanum tuberosum L.*). Gene 303:77–87. https://doi.org/10.1016/S0378-1119(02)01147-2
Tso TC (1990) Production, physiology, and biochemistry of tobacco plant. Ideals, Inc. Beltsville, Maryland, pp 322–323
Tso TC, Chaplin JF, Adams JD et al (1983) Simple correlation and multiple regression among leaf and smoke characteristics of burley tobaccos. Beitr Tabakforsch Intl 11(03):141–150
Tuberosa R (2012) Phenotyping for drought tolerance of crops in the genomics era. Front Physiol 3:347. https://doi.org/10.3389/fphys.2012.00347
Tuberosa R, Sanguineti MC, Landi P et al (2002) Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Mol Biol 48:697–712
Turner NC (1986) Adaptation to water deficits: a changing perspective. Aust J Plant Physiol 13:175–190
United Nations (2019) Goal 13: take urgent action to combat climate change and its impacts. https://www.un.org/sustainabledevelopment/climate-change/. Accessed 31 Sept 2021

Upadhyay SK, Kumar J, Alok A et al (2013) RNA guided genome editing for target gene mutations in wheat. G3 (Bethesda) 3:2233–2238
Vajpayee P, Tripathi RD, Rai UN, Ali MB, Singh SN (2000) Chromium accumulation reduces chlorophyll biosynthesis nitrate reductase activity and protein content in *Nympaea alba* L. Chemosphere 41:1075–1082
Van Ooijen JW (2006) Joinmap® 4, Software for the calculation of genetic linkage maps in experimental population. Plant Research International, Wageningen
Van Ooijen JW, Voorrips RE (2001) JoinMap® version 3.0: software for the calculation of genetic linkage maps. Plant Research International. Wageningen
Vardi V, Venkatrayulu C (2019) Assessment of heavy metals in water of selected places of Nellore coast of Andhra Pradesh through atomic absorption spectroscopy. Intl J Life Sci. https://doi.org/10.21276/SSR-IIJLS2019553
Varshney RK, Bohra A, Yu J et al (2021) Designing future crops: genomics-assisted breeding comes of age. Trends Plant Sci 26(6):631–649. https://doi.org/10.1016/j.tplants.2021.03.010
Varshney RK, Terauchi R, McCouch SR (2014) Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. PLoS Biol 12(6):e1001883. https://doi.org/10.1371/journal.pbio.1001883
Verma S, Yadav S, Singh I (2010) Trace metal concentration in different Indian tobacco products and related health implications. Food Chem Toxicol 48:2291–2297
Viswanath V, Strauss SH (2010). Modifying plant growth the cisgenic way. Inform Syst Biotechnol. News Rep 2010:1–4
Wagner GJ (1991) Secreting glandular trichomes: more than just hairs. Plant Physiol 96:675–679
Wang H, Zhao P, Hölscher D et al (2012) Nighttime sap flow of *Acacia mangium* and its implications for nighttime transpiration and stem water storage. J Plant Ecol 5(3):294–304
Wang RW, Cai X, Hu S et al (2020) Comparative analysis of the mitochondrial genomes of *Nicotiana tabacum*: Hints toward the key factors closely related to the cytoplasmic male sterility mechanism. Front Genet 11:257. https://doi.org/10.3389/fgene.2020.00257
Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 28:1–14
Wang X, Bennetzen JL (2015) Current status and prospects for the study of *Nicotiana* genomics, genetics, and nicotine biosynthesis genes. Mol Genet Genom 290(1):11–21. https://doi.org/10.1007/s00438-015-0989-7
Wang X, Yang S, Chen Y et al (2018) Comparative genome-wide characterization leading to simple sequence repeat marker development for *Nicotiana*. BMC Genomics 19(1):500. https://doi.org/10.1186/s12864-018-4878-4
Wang Y, Jiang J, Zhao X et al (2006) A novel LEA gene from *Tamarix androssowii* confers drought tolerance in transgenic tobacco. Plant Sci 171(6). https://doi.org/10.1016/j.plantsci.2006.06.011
Wang Y, Lv H, Xiang X et al (2021) Construction of a SNP fingerprinting database and population genetic analysis of cigar tobacco germplasm resources in China. Front Plant Sci. https://doi.org/10.3389/fpls.2021.618133
Wang Y, Sun C, Xu C (2016) Preliminary experiments on nano-silver against tobacco mosaic virus and its mechanism. Tob Sci Technol 49:22–30
Wania SH, Kumar V, Shriram V et al (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J 4:162–176
Waugh R, Jannik JL, Muehlbauer GJ et al (2009) The emergence of whole genome association scans in barley. Curr Opin Plant Biol 12:218–222. https://doi.org/10.1016/j.pbi.2008.12.007
Wei W, Zhang Y, Han L, Guan Z, Chai T (2008) A novel WRKY transcriptional factor from *Thlaspi caerulescens* negatively regulates the osmotic stress tolerance of transgenic tobacco. Plant Cell Rep 27(4):795–803
Weil C, Schaart J, Niks R et al (2010) Traditional plant breeding methods. Wageningen UR Plant Breeding report 338, Wageningen

Wenping LI, Sabaghnia N, Ranjbar R et al (2009) Correlation and path coefficient analysis and euclidean distance clustering for several characters in tobacco germplasm resource. Chin Tob Sci 30:59–63

Wilson D (1975) Stomatal diffusion resistances and leaf growth during droughting of *Lolium perenne* plants selected for contrasting epidermal ridging. Ann Appl Biol 79(1):83–94

Worland AJ, Gale MD, Law CN (1987) Wheat genetics. In: Lupton FJH (ed) Wheat breeding: its scientific principals. Chapman and Hill, London

Wu FN, Eannetta NT, Xu Y et al (2010) COSII genetic maps of two diploid *Nicotiana* species provide a detailed picture of synteny with tomato and insights into chromosome evolution in tetraploid *N. tabacum*. Theor Appl Genet 120(4):809–827. https://doi.org/10.1007/s00122-009-1206-z

Wu FN, Eannetta NT, Xu YM et al (2009) A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus *Capsicum*. Theor Appl Genet 118:1279–1293

Xi L, Xiong S, Ren Y et al (2011) Virtual visualization system for growth of tobacco root. Paper presented at 4th conference on computer and computing technologies in agriculture (CCTA), Oct 2010, Nanchang, China

Xiang D, Yao Z, Yanhua LY et al (2017) Analysis on solanesol content and genetic diversity of Chinese flue-cured tobacco (*Nicotiana tabacum* L.). Crop Sci 57(2):847–855. https://doi.org/10.2135/cropsci2016.07.0568

Xiang XH, Wu XR, Chao JT et al (2016) Genome-wide identification and expression analysis of the WRKY gene family in common tobacco (*Nicotiana tabacum* L.). Yi Chuan 38(9):840–856

Xiao B, Yuntao TY, Ni LN et al (2015) SNP-based genetic linkage map of tobacco (*Nicotiana tabacum* L.) using next-generation RAD sequencing. J Biol Res (Thessalon) 22:11. https://doi.org/10.1186/s40709-015-0034-3

Xiao BG, Xu ZL, Chen XJ et al (2006) Genetic linkage map constructed by using a DH population for the flue-cured tobacco. Acta Tab Sin 12:35–40

Xu MH, Zheng MH, Liu GT (1998) Genetic diversity in cultivars of *Nicotiana tabacum* revealed by RAPD markers. J Agril Biotechnol 6:281–285

Yamori W, Evans JR, Von CS (2010) Effects of growth and measurement light intensities on temperature dependence of CO_2 assimilation rate in tobacco leaves. Plant Cell Environ 3:332

Yang BC, Xiao B, Chen XJ et al (2007) Assessing the genetic diversity of tobacco germplasm using inter simple sequence repeat and inter-retrotransposon amplification polymorphism markers. Ann Appl Biol 150:393–401

Yang BC, Xiao BG, Chen XJ et al (2005a) Genetic diversity of flue-cured tobacco varieties based on ISSR markers. Hereditas (beijing) 27(5):753–758

Yang L, Li J, Ji J et al (2016) High temperature induces expression of tobacco transcription factor NtMYC2a to regulate nicotine and JA biosynthesis. Front Physiol. https://doi.org/10.3389/fphys201600465

Yang LY, Shuang LY, Jun YL et al (2018) Effects of different growth temperatures on growth development and plastid pigments metabolism of tobacco (*Nicotiana tabacum* L) plants. Bot Stud 59 (5). https://doi.org/10.1186/s40529-018-0221-2

Yang S, Li D, Fisher AJ (2019) CORESTA guide no. 16 molecular markers in tobacco. CORESTA, France

Yang X, Feng Y, He Z, Stoffell PJ (2005b) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353. https://doi.org/10.1016/jjtemb200502007

Yoshida S (2003) Molecular regulation of leaf senescence. Curr Opin Plant Biol 1:79–84

Young ND, Tanksley SD (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet 77(1):95–101

Yu YW, Xia YS, Li RH (2014) Association analysis of tobacco specific nitrosamines content in tobacco germplasm. Acta Tab Sin 20:48–55

Yuan F, Lyv MJ, Leng BY et al (2015) Comparative transcriptome analysis of developmental stages of the *Limonium bicolor* leaf generates insights into salt gland differentiation. Plant Cell Environ 38:1637–1657

Yuhe S (2012) BAC library construction of villi tobacco (*Nicotiana tomentosiformis*). Chin Tob Sci. Corpus ID: 87250212

Zeba N, Isbat M (2011) Multivariate analysis for yield and yield contributing traits in F0 and F1 generations in tobacco (*Nicotiana tabacum*). J Exp Biosci 2(1):101–106

Zhang DM (1994) Study on surface structure scanning of some tobacco seeds with electrical-glass observing. Chin Tob Sin 2:2–15

Zhang HY, Liu XZ, He CS et al (2005) Random amplified DNA polymorphism of Nicotiana tabacum L. cultivars. Biol Plant 49:605–607

Zhang HY, Liu XZ, He CS et al (2008) Genetic diversity among flue-cured tobacco cultivars based on RAPD and AFLP markers. Braz Arch Biol Technol 51(6):1097–1101. https://doi.org/10.1590/S1516-89132008000600003

Zhang JS, Wang RG, Yang CY et al (2012) Genetic diversity of agronomic traits and association analysis with SRAP markers in flue-cured tobacco (*Nicotiana tabacum*) varieties from China and abroad. Acta Agron Sin 38:1029–1041

Zhang Y, Zhang F, Li X et al (2013) Transcription activator-like effector nucleases enable efficient plant genome engineering. Plant Physiol 161(1):20–27. https://doi.org/10.1104/pp.112.205179

Zhou ZS, Huang SQ, Guo K et al (2007) Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativa* L. J Inorg Biochem 101:1–9

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. Plant Genome 1:1–20. https://doi.org/10.3835/plantgenome2008.06.0007ed

Zhu JK (2003) Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6(5):441–445

Zhu XG, Long SP, Ort DR (2010) Improving photosynthetic efficiency for greater yield. Ann Rev Plant Biol 61(1):235–261

Zimmerman JL, Goldberg RB (1977) DNA sequence organization in the genome of *Nicotiana tabacum*. Chromosoma 59:227–252

Chapter 11
Breeding for Abiotic Stress Resistance in Yam (*Dioscorea* Spp.) Using Biotechnology Approaches: Present Practices and Prospects

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Abstract Yam (*Dioscorea* spp.) is an important crop providing food, income and medicine in tropics and subtropics. Major yam species in West Africa such as *D. alata* and *D. rotundata* are subject to several biotic and abiotic stresses throughout the cropping cycle and post-harvest periods, resulting in substantial tuber yield and quality trait losses. Abiotic stresses (heat, drought, low soil fertility, salinity, water-logging, etc.) are currently exacerbated by the climate change effects. Yet, limited efforts exist in screening and developing yam varieties for those abiotic stresses. This book chapter reviews efforts of yam breeding programs in addressing yield losses associated with abiotic stresses and discusses perspectives in use of biotechnological tools for accelerating the development of abiotic stress resistant yam varieties.

Keywords Yam breeding · Drought · Low soil fertility · Climate change · Integrated management strategies · Mitigation · West Africa

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11.1 Introduction

Yam belongs to the genus *Dioscorea* and represents an important food and cash crop for over 300 million people in tropical and subtropical regions (Alabi et al. 2019). This genus is made of ~600 species, among which only 11 (*D. alata*, *D. rotundata*, *D. esculenta*, *D. cayenensis*, *D. bulbifera, D. dumetorum, D. trifida, D. opposita*, *D. japonica*, *D. nummularia,* and *D. pentaphylla*) are cultivated for food and income (Wilkin et al. 2005; Govaertset al. 2007). Yam species are perennial herbaceous climbers but grown as annual crops for their starchy tubers or aerial bulbils to provide food, income, and folk medicine. Yam zone of West Africa, also referred to as African "yam belt", is a region from west Cameroon to central Côte d'Ivoire. It is made of six countries, including Nigeria, Ghana, Côte d'Ivoire, Benin, Togo and Cameroon (Table 11.1). This zone accounted for ~92.5% (67.2 million tons) of the world yam production in 2018 (FAOSTAT 2020). White Guinea yam (*D. rotundata* Poir.), water yam (*D. alata* L.), and yellow yam (*D. cayenensis* Lam.) account for more than 95% of yam production worldwide. Yam tuber is an important source of nutrients (Omohimi et al. 2017) with low glycemic index, which gives better protection against diabetes and obesity (McKoy et al. 2014; Ijato and Tedela 2015). Yam production is also a major income generating activity for millions of smallholder farmers in West Africa; ~5 million people directly depend on its value chain for income (Azeteh et al. 2019; Mignouna et al. 2020). Thus, it provides an opportunity for poverty alleviation and food security. Besides, yam price in local

Table 11.1 Importance and distribution of yam production in the Africa yam belt countries

Countries	World rank	Region	Contribution (%)	Yam production (MT)	Cultivated lands (Million ha)	Yield (t ha^{-1})
Nigeria	1	West Africa	65.5	47.5	6.0	7.9
Ghana	2	West Africa	10.8	7.9	0.5	17.5
Côte d'Ivoire	3	West Africa	10.0	7.3	1.3	5.5
Benin	4	West Africa	4.1	2.9	0.2	13.6
Togo	6	West Africa	1.2	0.9	0.1	9.2
Cameroon	7	Central Africa	0.9	0.7	0.1	11.5

* Data retrieved from FAOSTAT database (2020). During the same period, the world production was 72.6 MT, the yield was 8.4 t ha^{-1}, and the total global cultivated area was 8.7 million ha. MT = Million tons. White Guinea yam (*D. rotundata* Poir.) is predominant in the African yam belt, except in Côte d'Ivoire where *D. alata* L. is most valued for cultivation and for food consumption (Bakayoko et al. 2021)

markets is less affected by international trade crises as it is often the case for cereals, and, thus it contributes to regulating the food system and selling prices. Although yam production in Africa is 40% that of cassava, its monetary value exceeds all other African staple crops and is equivalent to the combined value for the top three cereal crops (maize, rice and sorghum) (FAOSTAT 2020)

In the last two decades (1998–2018), yam production in West Africa doubled from ~34 to 67 million tons. This increase was largely attributed to the rapid expansion of cultivated lands from 3.6 to ~8.1 million hectares (Fig. 11.1, FAOSTAT 2020). At the same time, yam yield decreased from 9.6 to 8.2 t ha^{-1} (FAOSTAT 2020). The leading yam producers and corresponding production statistics are presented in Table 11.1. The current extensive yam farming and the search for new fertile lands by farmers will soon reach the limit due to rapid population growth. Besides, expanding cultivated lands is often associated with deforestation and natural resources degradation (McMahon 2013), which could exacerbate the effects of climate change in the region. It is noteworthy that West Africa has the fastest growing population in the world; with a five-fold increase since 1950 (73 to ~405 million in 2020) and the current population is projected to increase by three-fold until 2050 (Defrance et al. 2020). As a consequence, there will be an expected double food demand in sub-Saharan Africa by 2050 (CGIAR 2016; Thiele et al. 2017; Friedmann et al. 2018).This gap in food demand and supply balance is likely to be exacerbated by the climate change which is expected to raise temperatures, water shortages, pests and diseases (McMahon 2013; Godfray 2014). This situation is particularly alarming in West Africa where all staple food crops including the roots, tubers and banana crops will be affected negatively (Jarvis et al. 2012; Thiele et al. 2017).

Several biotic and abiotic constraints are currently responsible for the yam low yields in West Africa. These include poor soil fertility, use of low yielding varieties, pests and diseases, untimely access to quality seeds and research neglect (Azeteh et al. 2019; Darkwa et al. 2020a). Besides, the yam marketing system is poorly organized in most West African countries, and thus, hinders farmers from reaping optimum benefit from their activity (Azeteh et al. 2019). There are two ways in which

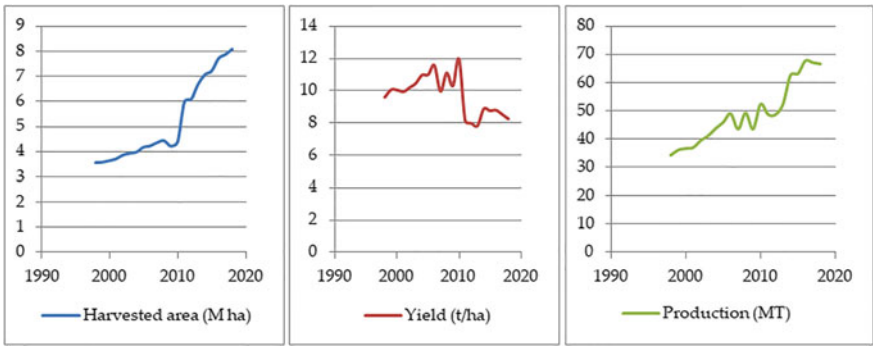

Fig. 11.1 Yam cultivated lands, tuber yield and production trends in West Africa (1998–2018; FAOSTAT 2020)

Fig. 11.2 Yam markets in West Africa

farmers can increase food production; either increase cultivated lands or increase the crop productivity by controlling the above-described production-limiting factors (Bradshaw 2017). Yam farmers in West Africa adopted the first way for the last two decades as the cultivated lands increased by a factor of 2.3 while yield decreased by ~15%, an approach that is ecologically unsustainable. There is, however, potential to increase food production by closing the yield gap on already cultivated lands in Africa (Bradshaw 2017). Pretty et al. (2011) showed a two-fold increase across 20 African countries by combining the use of new and improved varieties with changes to agronomic management since 1990s (Fig. 11.2).

Widespread resource-poor farmers in West Africa often find the use of external farm inputs costly in managing abiotic stresses such as drought, low soil fertility, etc. Disseminating improved yam varieties among smallholder farmers is, therefore, one of the most cost-effective and practical approaches in coping with these yield-limiting factors in resource-poor farms; as no additional investment is often required from farmers.

Plant breeding uses both sexual and asexual reproduction mechanisms in developing new yam varieties (Mondo et al. 2020). The first consists of an artificial hand- or natural open-pollination for the generation of full-sib and/or half-sib populations. Clones of selected progenies are then advanced for yield performance and stability over generations across multiple locations (Darkwa et al. 2020a; Norman et al. 2020). Despite advances reported in the molecular breeding field, yam improvement programs are still relying on conventional breeding approaches which are, however, less accurate, time-consuming and labor-intensive (Darkwa et al. 2020a). Efforts are being made to integrate biotechnology tools in yam breeding programs. These approaches include next-generation sequencing-based genotyping procedures, transcriptomics, metabolomics, genetic transformation (or transgenics), gene editing, marker-assisted selection, ploidy analysis, etc. (Darkwa et al. 2020a). These approaches aimed at shortening the breeding cycle, optimizing the breeding program as well as fast developing yam varieties to meet end-user' preferences (Tamiru et al. 2017; Friedmann et al. 2018; Darkwa et al. 2020a). These approaches have, however, been mainly used for yield and quality traits, less attention being attached to abiotic

Table 11.2 Yield losses associated with major biotic and abiotic factors in yam production

Factors	Species	Yield loss (%)	Distribution	References
Yam mosaic virus	*D. rotundata*	40–50	West Africa	Adeniji et al. (2012)
Anthracnose	*D. alata*	80–90	Worldwide	Penet et al. (2016)
Tuber rots	*D. rotundata*	25–40	West Africa	Bonire (1985); Acholo et al. (1997)
Nematode	*D. rotundata*	~40	West Africa	Atu et al. (1983); Kolombia et al. (2017)
Drought + heat	*D. alata*	18–33	West Africa	Srivastava et al. (2012)
Low soil fertility	*D. rotundata*	33–70	West Africa	Matsumoto et al. (2021)
Waterlogging	*D. alata, D. rotundata*	~57	West Africa	Igwilo and Udeh (1987)

stresses such as drought, low soil fertility, waterlogging, etc. although these are among major factors limiting yield in yam production systems (Table 11.2).

This book chapter reviews efforts of yam breeding programs in addressing yield losses associated with abiotic stresses and discusses perspectives in use of biotechnological tools in accelerating development of abiotic-resistant varieties.

11.2 Genetic Diversity and Sources of Resistance Genes for Abiotic Stress Breeding

The world checklist in Royal Botanic Gardens, Kew includes 644 accepted species for the family Dioscoreaceae, in the order Dioscoreales. These species are from five genera: *Dioscorea, Rajania, Tacca, Stenomeris* and *Trichopus* (Govaerts et al. 2007). Yams belong to the genus *Dioscorea* L., which is by far the largest genus in the family Dioscoreaceae. This genus is made of ~600 species, and thus constitutes ~95% of the family member species (Govaerts et al. 2007; Govaerts and Wilkin 2017). The genus *Dioscorea* is widely distributed in the tropical and temperate regions and occurs in diverse environments from forests to grasslands (savannah) (Wilkin et al. 2005).

This genus is in turn subdivided into five sections based on gross morphological characters, under which the various species are classified. The section *Enantiophyllum* is the largest in terms of number of species, and includes the most important species such as *D. alata*, *D. rotundata* and *D cayenensis.* Other members of this section are *D. opposita* Thunb., *D. japonica* Thunb., and *D. transversa* R.Br. (Bai and Ekanayake 1998). Members of this section are characterized by vines twining to the right, i.e., in clockwise direction when viewed from the ground upwards (Bai and Ekanayake 1998). The other sections are *Lasiophyton* (*D. dumetorum* (Kunth) Pax

and *D. hispida* Dennst.), *Macrogynodium* (*D. trifida* L.), *Combilium* (*D. esculenta* (Lour.) Burkill) and *Opsophyton* (*D. bulbifera* L.) which are characterized by vines twining to the left (Onwueme and Charles 1994; Bai and Ekanayake 1998). Among cultivated yam species, *D. alata*, *D. batatas* Decne or *D. opposita* and *D. esculenta* originate from Asia; *D. nummularia* Lam. and *D. pentaphylla* L. are from both Oceania and Asia; *D. trifida* is of American origin while *D. rotundata*, *D. cayenensis*, *D. bulbifera* and *D. dumetorum* are from Africa (Arnauet al. 2010). Out of the 11 cultivated yam species, *D. rotundata, D. cayenensis, D. alata,* and *D. trifida* are by far the major grown species worldwide, while the others are often referred to as minor ones. In addition to these cultivated species, there are semi-domesticated and wild species such as *D. burkilliana* J. Miège, *D. minutiflora* Engl., *D. praehensilis* Benth., *D. schimperiana* Hochst. Ex Kunth., *D. semperflorens* Uline, *D. mangenotiana* J. Miège, *D. smilacifolia* De Wild. & T. Durand, etc., that are grown on a subsistence basis or collected from the wild to fill the hunger gap during drought and lean periods (Adewumi et al. 2021). Recent phylogenetic analyses provided a large-scale phylogenetic tree containing 183 species and proposed dividing *Dioscorea* into two subgenera (*Dioscorea* and *Helmia*), with 11 major clades and 27 sections/species groups (Noda et al. 2020).

This huge interspecific diversity presents a potential for yam breeding programs by introgression of genes, mainly those for disease resistance and adaptation traits. However, there are serious cross-pollination barriers among species mainly due to differences in ploidy status, reproduction nature (dioecy) and evolutionary divergence in domestication process (Mondo et al. 2020). Yam species are characterized by diploids ($2n = 2x = 40$); triploids ($2n = 3x = 60$) and tetraploids ($2n = 4x = 80$) and most of these species are obligate outcrossers due to dioecy (separate male and female plant individuals). Nevertheless, controlled and spontaneous hybridizations among some of the related species have been reported by several researches in Africa and Asia (Scarcelli et al. 2006; Lebot et al. 2019a; Mondo et al. 2020). Methods of breaking hybridization barriers among species and cultivars with different ploidy levels have been developed by the CIRAD-Guadeloupe (Abraham et al. 2013). An extensive discussion on how cross-pollination success can be improved for yam has been reviewed by Mondo et al. (2020). Use of biotechnological approaches such as ploidy manipulation, gene editing, androgenesis or gynogenesis induction, embryo rescue, somatic and protoplast fusion, etc. to break intra and interspecific cross pollination barriers has been advocated.

In addition to high diversity in terms of species, there are several cultivars within each yam species, and whose names vary greatly with local and national languages as well as their source. This makes challenging the assessment of local landraces diversity, as more often, different names may refer to the same cultivar or a single name allocated to several cultivars (Mignouna et al. 2002a; Azeteh et al. 2019; Kouakou et al.2019; Agre et al. 2021; Bakayoko et al. 2021). Therefore, more elaborate and robust diversity studies are necessary in the West Africa for effective determination of the genetic diversity of yam within regions to facilitate the conservation efforts and use of existing variability in the crop improvement programs. Several assessment methods are used to determine the genetic diversity among yam accessions. These

include morphological, biochemical/isoenzymes, and molecular markers (Loko et al. 2017; Agre et al. 2019, 2021; Nkhoma et al. 2020). Morphological and biochemical markers are widely used to assess genetic diversity in plants. However, they are limited in number and highly influenced by environmental factors and plant developmental stages (Stanley et al. 2020; Nkhoma et al. 2020; Agre et al. 2021). Molecular markers represent an alternative for estimating genetic diversity. They are stable, polymorphic, readily available in the genome, reproducible, dominant/codominant, and not sensitive to environmental factors and the plant growth stages (Nkhoma et al. 2020). Molecular markers previously used for yam genetic diversity studies include random amplified polymorphic DNA (RAPD) (Asemota et al. 1995), amplified fragment length polymorphism (AFLP) (Mignouna et al. 1998; Terauchi and Kahl 1999; Mignouna et al. 2002a, b), simple sequence repeat (SSR) (Arnau et al. 2009; Loko et al. 2017; Mulualem et al. 2018), inter-simple sequence repeat (ISSR) (Ousmael et al. 2019), and single nucleotide polymorphism (SNP) markers (Agre et al. 2019, 2021; Darkwa et al. 2020b; Bhattacharjee et al. 2020; Bakayoko et al. 2021). However, the reproducibility and reliability of markers like AFLP are limited, time-consuming, and characterized by low distribution across the genome. From the last decade, SNPs generated through the next generation sequencing approaches are the most widely used in yam diversity studies because of their stability and abundance in the genome (Girma et al. 2015; Akakpo et al. 2017; Siadjeu et al. 2018; Scarcelli et al. 2019; Darkwa et al. 2020b; Bhattacharjee et al. 2020; Agre et al. 2021).There is an interest in combining molecular and phenotypic data in diversity analyses (Agre et al. 2019, 2020, 2021; Darkwa et al. 2020b). The rationale is that genotypic and phenotypic data display very low or negligible correlations and produce non-duplicate information since a large portion of variation detected by molecular markers is non-adaptive compared with phenotypic characters which are influenced by the environment (Agre et al. 2021).

In addition to challenges in diversity assessment, there is a threat on genetic diversity due the genetic erosion since conservation initiatives are often absent, poorly organized or lack running funds in most West Africa countries. For instance, although elite species such as *D. rotundata, D. alata, D. cayenensis, D. bulbifera,* and *D. dumetorum* are available all over in West Africa, the minor ones like *D. esculenta, D. trifida, D. liebrechtsiana,* and *D. schimperiana*are at high risk and are increasingly rare (Azeteh et al. 2019). Research and maintenance of existing diversity is still weak in most of the West African countries. Besides, farmers only maintain the genotypes suitable to their needs and, therefore, accelerate the genetic erosion in most countries (Adewumi et al. 2021). Furthermore, only on-farm conservation is made in most countries without any back-up in the form of *in-vitro* culture or in cryopreservation, despite exposure of conserved materials to environmental stresses which are exacerbated by climate changes (Azeteh et al. 2019; Adewumi et al. 2021). The few existing conservation initiatives often focus on cultivated species and neglect wild relatives which are, however, crucial in crop improvement programs. Since yam is mainly propagated using tubers; botanical seeds are only used for breeding purposes. This clonal propagation reduces the genetic diversity, leading to the vulnerability to plant

diseases and the difficulty of purging deleterious mutations from the germplasms (Sugihara et al. 2021).

The International Institute of Tropical Agriculture (IITA) is a member of the Consultative Group for International Agricultural Research (CGIAR) with the global mandate for yam research. In partnership with its national and international partners, IITA maintains, develops, and releases yam varieties to meet farmers' and other end-users' needs and demands. IITA maintains the largest collection of yams in the world. Its yam germplasm collections steadily increased from the 3,319 in 2010 to 5,788 in 2018 and from 8 to 10 species during the same period (IITA 2018). Most of the accessions were collected from West and Central Africa, and *D. rotundata* makes up about 68% of the total collection. All of the accessions are grown annually in the field, but 1,544 of these are also conserved in tissue culture as in vitro plantlets (IITA 2018).

11.3 Cytology and Genetics of Yam Species

11.3.1 Cytogenetics

Dioscorea is one of the most problematic genera for cytogenetic studies. The chromosome counting is difficult because of the small size of chromosomes as well as their tendency to stick together. Besides, their satellites are sometimes as large as the chromosomes themselves (Bousalem et al. 2006). Studies have revealed that the basic chromosome number of the three major cultivated yam species, *D. rotundata, D. alata,* and *D. trifida,* is $x = 20$ (Arnau et al. 2010). About 33% of *Dioscorea* species are polyploids, with a varying ploidy level (Sugihara et al. 2021), a consequence of previous hybridization events. Among cultivated ones, *D. rotundata* and its wild relatives (*D. abyssinica* and *D. praehensilis*) are dominated by diploids ($2n = 2x = 40$), *D. cayenensis* by triploid ($2n = 3x = 60$) males, *D. alata* and *D. trifida* possesses diploid, triploid, and tetraploid ($2n = 4x = 80$) clones. These chromosome numbers for major cultivated yams and their wild relatives were recently confirmed by a study combining genotyping-based, chromosome counting and flow cytometry ploidy determination methods in the IITA maintained core collection (Gatarira et al. 2021). It is noteworthy that some *Dioscorea* sections such as *Stenophora* are characterized by a basic chromosome number $x = 10$ (Sugihara et al. 2021).

Triploid and tetraploid cultivars tend to be more vigorous, stress-resistant and higher yielding than diploid counterparts (Arnau et al. 2010). Besides, there are reports demonstrating an association between the ploidy level, fertility, and the flower sex in yam, such that all triploids express either male or non-flowering behavior as opposed to their diploid and tetraploid counterparts (Abraham and Nair 1991; Girma et al. 2014, 2019; Mondo et al. 2020). Babil et al. (2016) showed the effectiveness of in vitro polyploidy induction for obtaining polyploid variants for use as genetic resources in *D. rotundata* breeding.

11.3.2 Yam Breeding Objectives

Yam breeding is relatively recent compared to other root and tuber crops such as potato and cassava. It started in 1960s with *D. trifida* in the Caribbean (Degras 1969). Breeding works on *D. rotundata* in Nigeria (Sadik and Okereke 1975) and *D. alata* in India (Abraham et al. 1986) started in the 1970s and 1980s, respectively. The effectiveness of these works was seriously affected by insufficient knowledge on the origin, diversity and genetics of yam species. With understanding of yam cytology and floral biology; hybridization (intra and interspecific crosses), cytogenetic and mutation techniques, in vitro culture and molecular breeding were gradually introduced (Arnau et al. 2010; Darkwa et al. 2020a). Although varying with regional priorities and the species involved, the main yam breeding objectives are:

- High and stable tuber yield,
- Good tuber quality including flesh oxidation rate (stable color of the cut tuber), taste, texture, dry matter content, etc.
- Tuber characteristics that facilitate harvesting and meet consumers needs (size, shape, culinary quality),
- Plant architecture (e.g. semi-dwarf) that reduces the need for staking,
- Resistance to abiotic (drought and low soil nutrients) and biotic stresses (virus, fungi, rots and nematodes).

These breeding objectives change over time and are influenced by farmers' and other end-users' local/regional preferences. Local trait preferences include early maturity or tuber bulking; less susceptible to in soil deformation; long shelf-life of fresh tubers or processed food products; and high levels of culinary attributes suited to consumer needs for fresh and processed yam. Unfortunately, most of these traits are still missing in released varieties, and thus, explaining their low market penetration (Darkwa et al. 2020a). To boost the adoption of new varieties, focus of yam breeding programs in West Africa should be led by farmers' and other end-users' expectations.

11.3.3 Methods and Techniques in Yam Breeding

The complexity of yam reproductive biology, the expected low research investment returns, and its regional importance have limited attention to yam breeding, a crop which is still regarded as an orphan crop. The reproductive biology complexity lies mainly on its low and erratic flowering behavior as a result of continuous vegetative propagation following its domestication (Mondo et al. 2020). Besides, there are disproportionately low female-to-male sex ratio, poor synchronization of flowering periods between males and females, low pollen viability, low stigma receptivity, low fruit and seed set, and low seed viability (Lebot et al. 2019a; Agre et al. 2020; Darkwa et al. 2020a; Mondo et al. 2020, 2021a, b, c). Before understanding its cytology, some

species of cultivated yam such as *D. alata* were thought to be completely sterile, and thus, unable to undergo hybridization (Arnau et al. 2010). Efforts are ongoing at IITA to address constraints to yam sexual reproduction. These efforts include establishing cost-effective protocols for quality pollen collection and storage; agronomic and hormonal manipulations to induce profuse flowering; determination of better pollination time and practices; profiling of genotypes for ploidy status and development of SNP markers to predict sex identity and cross-pollination success (Mondo et al. 2020, 2021a, b, c; Gatariraet al. 2021). Further initiatives are needed such as the adoption of biotechnological approaches (ploidy manipulation, gene editing, androgenesis orgynogenesis induction, embryo rescue, somatic and protoplast fusion, etc. to break intra and interspecific cross-compatibility barriers); proper site selection, training of pollinators, rearing of insect pollinators, etc.

Several breeding methods and techniques are used in yam improvement, including domestication of wild species, introduction and selection of clones, hybridization (intra and interspecific crosses), cytogenetic and mutation techniques, in vitro culture and molecular breeding (Arnau et al. 2010; Darkwa et al. 2020a; Asfaw et al. 2021). Improved varieties are superior for most traits compared to local landraces in West Africa. They are uniform and early sprouting, have better crop establishment, good vigor, higher survival rate and high tuber yields (a yield advantage of approximately 50%) and superior tuber dry matter and quality (Darkwa et al. 2020a).

Despite its limitations, the conventional breeding is the major contributor of improved yam cultivars released in West Africa. A collaborative research between IITA, National Root Crops Research Institute (NRCRI, Umudike, Nigeria) and the Crops Research Institute of Ghana (CRIG) developed and released 15 *D. rotundata* clones in Nigeria and two in Ghana for the period of 2001–2016. With the venue of the Africa Yam Project, further varieties have been released in beneficiary countries: Nigeria, Ghana, Côte d'Ivoire and Benin. The major characteristics of these released varieties are described in Table 11.2 of the chapter on biotic stress breeding in yam.

Up to date, no improved yam variety using molecular tools has been reported (Darkwa et al. 2020a). Advances achieved in incorporating molecular markers in yam breeding programs in West Africa are discussed in Sect. 11.4 of this chapter.

11.3.3.1 Yam Breeding Scheme

The yam breeding program uses both botanical seed (sexual reproduction) and tubers (vegetative propagation) during new variety development process (Figs. 11.3, 11.4 and 11.5, Mondo et al. 2020). Sexual reproduction involves an artificial hand or natural open pollination during flowering to generate genetically variable offspring for selection. It starts with the collection of desirable parents for cross-combinations. This selection of parents is based on information from characterization studies and considers diverse aspects: agronomic traits (including biotic and abiotic stress tolerance), tuber quality, ploidy status, flowering ability, trait-combination ability, etc. (Arnau et al. 2010; Lebot et al. 2019a).

Fig. 11.3 Yam inflorence: **a** a profuse male plant, **b** spike with male flowers, **c** spike with female flowers

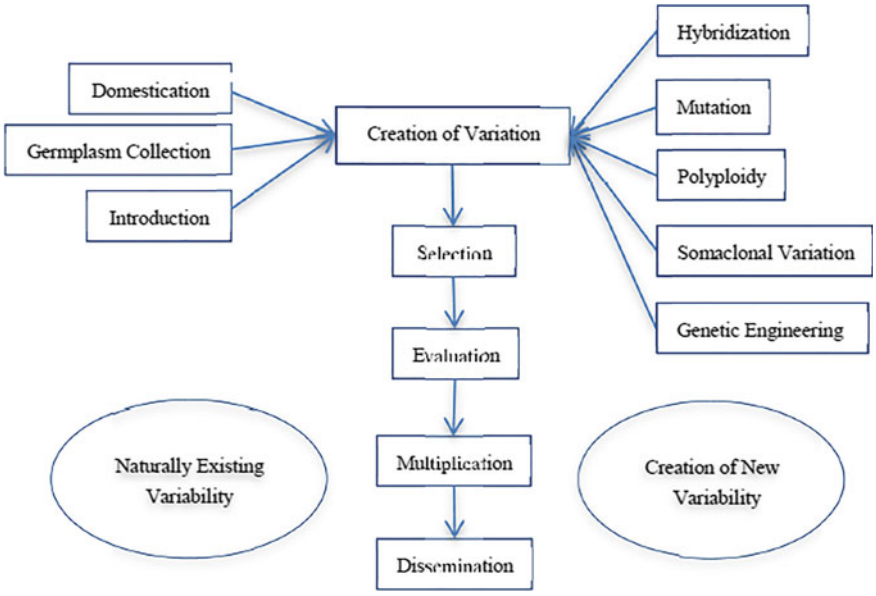

Fig. 11.4 Simplified yam breeding scheme (Adapted from Otoo 2017)

Yam species are mostly dioecious; they produce distinct male or female flower on separate plants (Tamiru et al. 2017; Agre et al. 2020; Mondo et al. 2020).Thus, separate hybridization blocks of male and female parents are necessary for cultivars with related flowering dates in case the hand pollination is planned. The female flowers are bagged for 2–7 days before the flower opens, depending on the spike length. They are hand-pollinated and then bagged for two weeks (Darkwa et al. 2020a; Mondo et al. 2020). Hybridization by hand pollination is the best way to ensure biparental crossing when the parental flowering dates do not coincide or if the male parent does not flower during a particular year (Arnau et al. 2010). The purity of crosses is ensured by planting biparental combinations in isolated plots. Although not systematically specified, separation distance between crossing blocks usually exceeds 500 m. The same isolation distance is recommended between crossing blocks and forest environments to prevent unwanted pollen source. However, executing planned and controlled crosses are often constrained by the tiny size of flowers, the availability of fertile female and male plants, and the short pollination window due to partial asynchrony (Lebot et al. 2019a; Norman et al. 2020). Half-sib breeding, which entails the random open-pollination among desirable parents, is a viable alternative to the full-sib breeding which utilizes planned and controlled artificial pollination (Norman et al. 2018). When open-pollination is desired, male and female individual plants are grown close to each other (1 m $\times$ 1 m) and their vines staked to same supports, helping the floral branches to mix and pollination to be carried out by thrips to generate polycross seeds (Arnau et al. 2010; Mondo et al. 2020). Open pollination polycross design is cost-effective and convenient especially when using fertile parents; although the male parent of the progenies is usually unknown (Norman et al. 2020). Norman et al. (2020) showed the effectiveness of SNP markers in reconstructing parentage of offspring from polycross blocks. Multiple planting dates are advised to increase chances of synchronization of the flowering of male and female parents.

In addition to asynchronized flowering between males and females, the male flower life cycle is very short (3–4 h) and usually open at noon. Therefore, the indicated moment for pollination is during the noon hours (Arnau et al. 2010). However, due to harsh environmental conditions, yam pollination is rarely performed at noon hours. Technicians most conveniently work in crossing blocks from 8 a.m. to 12 noon. Studies are undergoing at IITA to assess differences in pollination success with time of pollination to design measures of raising the outcome during the most convenient hours of the day. Based on past experiences, the female flowers are receptive for up to 10 days (depending on prevailing weather conditions) (Abraham and Nair 1990; Arnau et al. 2010; Mondo et al. 2020). A way of enhancing pollen viability cycle is to open the male flowers 1 h before their natural opening to take out the anthers for deposit on the stigmatic surface (Arnau et al. 2010).

At physiological maturity when fruits are dry, botanical seeds from controlled biparental crosses or open-pollinations are collected before they disperse from the capsules, processed and stored under room temperature for ~3 to 4 months until the end of dormancy (Abraham 1992). The next season, the botanical seeds are sown in nursery beds or seedling trays filled with suitable growing media such as

carbonized rice husk and coco peat mixed with sterilized top soil (Norman et al. 2021). Seed germination starts in 10 days after sowing and continues for one month (Darkwa et al. 2020a; Norman et al. 2021). The seedlings are then transplanted to the pots under screen house or nursery beds in the field for single plant selections (Fig. 11.5). Next steps include tuber family evaluation, the second clonal generation evaluation, the preliminary performance trial, advanced multi-location and multi-season performance trial and on-farm variety validation trial for an official release and commercial deployment (Arnau et al. 2010; Darkwa et al. 2020a). Figure 11.5 describes the standard yam breeding cycle (Fig. 11.6).

Fig. 11.5 Major yam breeding phases: **a** hand pollination, **b** dry fruit processing, **c** botanical seed processing prior seedling nursery sowing, **d** seedlings germinating on nursery trays, **e** seedling transplantation in pots containing enriched medium, **f** seedlings management on pots, **g** tuber harvest from seedling nursery, **h** tuber family evaluation, **i** advanced breeding lines under evaluation and **j** seed multiplication prior release

Fig. 11.6 Aerial view of yam crossing blocks at IITA, Ibadan, Nigeria

11.4 Marker-Assisted Selection and Major Milestones in Yam Breeding

11.4.1 Reference Genome Sequences

Advances and decreased cost in genome sequencing through next generation sequencing (NGS) technologies (such as genotyping-by-sequencing (GBS) and diversity array technology (DArT)) have enabled the generation of millions of novel markers and high-density genetic maps in major food crops (Thottathil et al. 2016), including yams (Tamiru et al. 2017; Bhattacharjee et al. 2018; Cormier et al. 2019; Darkwa et al. 2020a). Development of reference genome sequences facilitated understanding of the underlying genetics of complex traits in plants, the identification of key genes regulating important agronomic and adaptation traits as well as the detection of genetic variability among cultivars (Thottathil et al. 2016).

Tamiru et al. (2017) developed and released the first reference genome sequence of *D. rotundata* accession TDr96_F1using flow cytometry and *k*-mer analyses of genome sequences. *Dioscorea rotundata* genome size is 594 Mb, contains 26,198 protein coding genes, out of which 76.4% are distributed among 21 linkage groups (http://genome-e.ibrc.or.jp/home/bioinformatics-team/yam). Recently, an updated version of the *D. rotundata* sequence (using long reads generated by Oxford Nanopore Technologies) has been released, covering a total of 636.8 Mb and distributed on 20 linkage groups of the genome with an N_{50} of 137,007 bp (Sugihara et al. 2020) (https://www.pnas.org/content/pnas/suppl/2020/12/02/2015830117.DCSupplemental/pnas.2015830117.sapp.pdf).

A genetic linkage map of *D. alata* was developed from 380 EST-SSRs on 20 linkage groups for the identification of QTLs controlling anthracnose disease resistance (Bhattacharjee et al. 2018). Cormier et al. (2019) established the first high-density genetic map of *D. alata* using genotyping-by sequencing (GBS). In this high-density map, 20 linkage groups were resolved and 1,579 polymorphic markers

were ordered. The consensus map length was 2,613.5 cM with an average SNP interval of 1.68 cM. This corresponded with estimated genome coverage of 94%, and thus, promoted further investigations on the inheritance of key traits and the development of molecular breeding tools. *Dioscorea alata* newly released chromosome-scale reference genome v2 is a dense 10 k marker consensus genetic linkage map from five populations involving seven distinct parental genotypes (Bredeson et al. 2021).The genome assembly spans a total of 479.5 Mb, consistent with estimates of 455 ± 39 Mb by flow cytometry, and 477 Mb by *k*-mer-based analyses.

Besides, a reference genome for *D. dumetorum* has been developed, and the assembly represents 485 Mbp of the genome with an N_{50} of over 3.2 Mbp (Siadjeu et al. 2020). A total of 35,269 protein-encoding gene models and 9,941 non-coding RNA genes were predicted, and functional annotations were assigned. Other yam species, such as *D. esculenta,* have also being sequenced and the reference genomes developed. *Dioscorea esculenta* total genome size is 153, 437 bp in length and demonstrates a typical quadripartite structure containing a large single copy (LSC, 83,628 bp) and a small single copy (SSC, 18,893 bp), separated by a pair of inverted repeats (IRa, IRb) of 25,458 bp (Chen et al. 2020). The establishment of these reference genome sequences in yam crop has opened a new avenue for exploiting and in-depth understanding of the yam genetics, genomics and domestication, essential for successful yam breeding (Darkwa et al. 2020a).

11.4.2 Molecular Markers Used in Yam Improvement Programs

Introduction of biotechnology tools and development of reference genomes had empowered breeders to use modern genetic tools such as molecular markers, thereby accelerating the release of improved varieties to farmers (Bredeson et al. 2021). Although no yam variety has yet been released using such modern tools, significant advances have been recorded in the process of fully implementing marker-assisted selection (MAS) in yam breeding (see review by Darkwa et al. 2020a). Past research in yam breeding used cytogenetic techniques and different types of markers (isozymes, RFLP, RAPD, AFLP, SSR and SNP) for genetic diversity studies, phylogenetic relationships, estimation of population structures, cultivar fingerprinting, mapping of major effect genes and QTLs, identification of elite genotypes in crop breeding programs, and for validation of progenies originating from genetic hybridizations (Darkwa et al. 2020a; Norman et al. 2020). It is noteworthy that early use of markers in yam research was mainly for diversity studies, parentage analysis, origin and phylogenetic studies, and identification of genes controlling major diseases such as yam anthracnose disease and yam mosaic virus (Arnau et al. 2010).

With the venue of the Africa Yam Project of IITA (in 2014), there was a shift from the predominance of genetic diversity studies to QTL analyses, GWAS, evolution and domestication of *Dioscorea* species. IITA and its partners are making substantial

efforts to develop diverse molecular markers both for Guinea and water yams (Tamiru et al. 2015, 2017; Cormier et al. 2019, 2021; Darkwa et al. 2020a; Norman et al. 2020; Mondo et al. 2021b; Bredeson et al. 2021). For instance, Tamiru et al. (2015) developed 90 SSR markers from an enriched genomic library of yellow Guinea yam (*D. cayenensis*) with the assumption that these SSRs could be successfully transferred to the two major cultivated species (*D. rotundata* and *D. alata*). A higher level of transferability to *D. rotundata* (94%) was reported due its proven relatedness with *D. cayenensis* (Dansiet al. 2013) while it was low with *D. alata* (57%). Besides, Africa Yam Project has made significant efforts to develop more genetic tools and genomic resources to transform yam breeding in West Africa. It developed markers for major traits such plant vigor and sex, flowering intensity, number of tubers per plant, tuber yield, flesh tuber oxidation, disease resistance (mostly YAD and YMV), tuber appearance, and spines on tuber surface. These markers are currently undergoing validation and conversion into kompetitive allele-specific PCR(KASP) markers prior their use for the MAS in yam breeding. Despite the challenge brought by abiotic stresses (drought, low soil fertility, flooding, etc.) which have been aggravated by changes in climate, limited efforts have been dedicated to abiotic resistance breeding or developing markers associated with their resistance/tolerance. The way forward in introducing abiotic stresses among breeding priorities in yam is discussed in the Sects. 11.5 and 11.6 of this book chapter.

11.4.3 Other Omics Tools Used in Yam Improvement

As stated earlier, yam breeding has gone tremendous transformation with the venue of Africa Yam Project in West Africa. Many novel molecular tools have been introduced in yam breeding: the next-generation-based genotyping procedures (GBS and DArT), transcriptome sequencing, and metabolomics (Darkwa et al. 2020a).

Genotyping-by sequencing (GBS) is a next-generation genotyping procedure which helps unraveling the magnitude of genetic similarity and diversity within and between cultivated species and their wild relatives (Spindel et al. 2013). The GBS procedure is based on minimizing genome complexity with restriction enzymes, coupled with multiplex next-generation sequencing for high-density SNP discovery (Elshire et al. 2011). Diversity Array Technology (DArT) is another next-generation-based genotyping procedure which is a robust and low-cost high-throughput open platform method for DNA polymorphism analysis. It provides high call rates and scoring reproducibility compared to other sequencing techniques. GBS and DArT have proven potential in exploring genetic diversity, evolution, population structure and identification of loci linked to important agronomic and quality traits in yam (Girma et al. 2014; Saski et al. 2015; Siadjeu et al. 2018; Agre et al. 2019, 2021; Cormier et al. 2019, 2021; Gatarira et al. 2020; Bhattacharjee et al. 2020; Mondo et al. 2021b).

Transcriptome sequencing uses genome-wide differential RNA expression to provide a better understanding of biological pathways and molecular mechanisms

that control important but complex traits in plants. Transcriptome sequencing allowed investigating gene expression by large-scale generation of ESTs from a susceptible (TDa95/0310) and two resistant (TDa87/01091 and TDa95/0328) water yam genotypes infected with the anthracnose (*C. gloeosporioides*) (Narina et al. 2011). Gene expression of flavonoid (purple flesh color) to characterize the transcriptomes of tubers from a purple-flesh and a white flesh variety of *D. alata* tubers is another application of transcriptome sequencing procedure (Wu et al. 2015). The Super SAGE transcriptome profiling identified flowering and sex-related genes in *D. rotundata* (Girma et al. 2019). A total of 88 tags were expressed in male, female and monoecious plants. Among these tags, 18 matched with genes for flower development and sex determination previously identified in many plant species. Siadjeu et al. (2021) used transcriptome sequence to reveal candidate genes involved in the post-harvest hardening of *D. dumetorum* and thus opened an avenue for improving the storability of this yam species.

Metabolomic techniques produce extensive biochemical phenotypes that can be indicative of quality traits. Desirable quality traits are often directly linked with metabolite composition, and thus, providing a path to metabolite-marker-based breeding (Bino et al. 2004; Fernie and Schauer 2009). This explains the increasing interest to metabolomics in complement to genomics in yam studies (Friedmann et al. 2019). Price et al. (2016, 2017, 2018) and Lebot et al. (2019b) are the most relevant reports on the application of metabolomic technology in yam breeding. Metabolite profiles provided enormous insight into biochemically related species and revealed *Dioscorea* species as potential sources of essential compounds such as shikimic acid (Price et al. 2016). In addition, a large number of unknown metabolites highlighted the understudied nature of genus *Dioscorea.* Price et al. (2017) identified a subgroup of metabolites useful for accurate species classification and emphasized the possibility of predicting tuber composition from leaf profiles. Metabolic differences were accession specific and usually confined to compound classes, and will therefore, support trait-targeting for metabolite markers. Price et al. (2018) investigated the cross-species carotenoid profiling of 46 yam accessions belonging to five species (*D. alata, D. bulbifera, D. cayenensis, D. dumetorum* and *D. rotundata*). They found non-significant differences between the *D. rotundata* and *D. alata* accessions on β-carotene content and provitamin A activity. Besides, they elucidated the absence of link between yellow tuber flesh color and provitamin A content in yam, as opposed to reports on cassava and sweet potato. A compound database and concentration range for metabolites detected in the major root, tuber and banana (RTB) crops, following metabolomics based diversity screening of global collections held within the CGIAR institutes have been elaborated (Price et al. 2020). They provide a valuable resource regarding the comparative biochemical composition of each RTB crop and highlight the potential diversity available for incorporation into crop improvement programs. Linking biochemical signatures with several agronomic and sensory characters offers potential to expedite the selection and consequently the breeding cycle. Lebot et al. (2019b) developed and optimized a high-performance thin-layer chromatography (HPTLC) protocol for the rapid quantification of individual sugars, allantoin, phenolic acids, catechins and saponins in yam tuber flours. This technique

was successfully used for the rapid quantification of compounds related to tuber flour quality of 522 accessions from eight *Dioscorea* species.

GWAS is another method used to identify and understand the genetic architecture of the genes responsible for complex traits, by exploiting linkage disequilibrium. In opposition to QTL analysis (which assays only allelic diversity that segregates between the parents), GWAS uses natural populations (collection of individual varieties or inbred lines), thus increases the power to dissect historical recombinations. This technology is currently ongoing at IITA (under Africa Yam and NSFBREAD projects), to determine QTLs linked to various traits in *D. rotundata* and *D. alata*, to facilitate marker-assisted breeding in yam (Gatarira et al. 2020; Mondo et al. 2021b; Agre et al. 2021).

In addition to above-described novel techniques, protocols for implementing genetic transformation (Zhu et al. 2009; Zhao-wei 2012; Nyaboga et al. 2014; Manoharan et al. 2016) and gene editing through the CRISPR/Cas9-based genome-editing system in *Dioscorea* species have been developed (Feng et al. 2018; Syombua et al. 2021). The tissue culture techniques for embryo rescue, rapid seed multiplication, and production of disease-free planting materials have become common practices in yam breeding programs and hold potential in accelerating breeding cycle and thus allows fast delivery of improved varieties to yam farmers (Aighewi et al. 2015).

11.5 Genetic Improvement for Abiotic Stress Tolerance in Yam

Yam is a very demanding crop in terms of soil nutrient as well as rainfall amount and distribution (Adifon et al. 2019; Neina 2021). Thus, the rainfed and low-input farming systems by West African resource-poor farmers are a likely source of stress since the crop requirements are seldom met (Frossard et al. 2017; Matsumoto et al. 2021). This gap in requirements will most probably be exacerbated by climate change and rapid population growth (Thiele et al. 2017). Agro-morphological characterization of popular yam varieties of Togo and Benin were performed for drought, low soil fertility, high soil moisture content, and insect pest tolerance (Dansi et al. 2013; Loko et al. 2015). Response of yam genotypes to soil fertility regimes in Côte d'Ivoire and Nigeria was also reported (Diby et al. 2009; Matsumoto et al. 2021). Limited efforts are made in breeding yam for abiotic stresses in West Africa. Although yam is subjected to several abiotic stresses (drought, heat, salinity, low soil fertility, acidity, waterlogging, etc.), this book chapter emphasizes drought and low soil fertility as the most important and widely distributed abiotic threats on yam production. It is noteworthy that the "emergence-tuber initiation" phase is more sensitive to environmental stresses than the "tuber initiation-harvest" phase and thus should necessitate special care (Neina 2021).

11.5.1 Breeding Yam for Drought and Heat Stress Tolerance

Yam grows well under 20–35 °C and requires ample and well-distributed moisture (>1500 mm) (Cornet 2005; Neina 2021). Climate change with more weather extremes (high temperature and erratic rainfall) affects yam performance in the mostly rainfed yam farming of West Africa (Srivastava et al. 2012). High temperatures affect the photosynthetic rate and metabolism related to dry matter accumulation, tuber initiation, and development in root and tuber crops (Thiele et al. 2017). Moisture stress delays tuber initiation in water yam (Onwueme 1975; Remesh et al. 2019). Growth constraints and yam yield reduction were reported in *D. alata* and *D. rotundata* under water stress conditions (Diby et al. 2009). Thiele et al. (2017) suggested that maintaining yields of root and tuber crops will require enhanced heat and drought tolerance, which are controlled by different physiological mechanisms and genetic pathways. Gao et al. (2018) showed drought tolerance in a *D. nipponica* accession. This accession used two coping strategies: drought avoidance (stomatal closure and transpiration reduction), and drought tolerance by osmotic adjustment. Liu et al. (2017) characterized and examined regulatory mechanisms of dioscorin genes and showed that *Da-dio5* from *D .alata* has a regulating role for three abiotic stresses, including high temperature, low temperature, and drought. No report on breeding for drought tolerance has been reported in West Africa due to a highly demanding phenotyping procedure requirement. In an earlier effort at IITA to identify yam clones with drought tolerance, Odoh (2014) evaluated the performance of 32*D. alata* and 49 *D. rotundata* genotypes under stress conditions in the glasshouse and field. Five genotypes of both *D. alata* and *D. rotundata* were identified as promising sources of genes for drought tolerance.

11.5.2 Breeding for Tolerance to Low Soil Fertility in Yam

Yam adapts to a range of soils, but it prefers friable, deep, loose, and textured loamy soil, which allows proper tuber expansion. It requires excellent soil drainage as it is less tolerant to waterlogging stress. For better yields, high soil fertility (mainly in nitrogen, potassium, magnesium, calcium, organic matter) is required (Table 11.3). Based on this soil fertility requirement, farmers usually grow yam first in crop rotation or newly opened land (Floquet et al. 2012; Frossard et al. 2017; Adifon et al. 2019; Hgaza et al. 2020; Neina 2021). Yam requires a pH ranging from 5 to 7. It is very sensitive to low pH in soils where the aluminum concentration is high (Diby et al. 2011; Frossard et al. 2017).

However, the soil requirements are seldom met in smallholder farms. As a result, farmers exploit a field for a time and leave it for a freshly opened one. This farming practice is not sustainable under the current demographic pressure context and presents environmental consequences. Cultivated land expansion is often associated with more deforestation and the risk of exacerbating climate change effects

Table 11.3 Estimated yam nutrient requirements (in kg ha^{-1})

Country	Species	N	K	P	Ca	Mg	S	Expected yield (t ha^{-1})	References
Côte d'Ivoire	*D. alata* *D. rotundata*	240	269	11	8.5	11	66	-	Diby et al. (2011)
Nigeria	*D. rotundata*	90	74	40	-	-	-	-	Dare et al. (2014)
Côte d'Ivoire	*D. rotundata*	120	130	10	-	-	-	25	Hgaza et al. (2020)
Côte d'Ivoire	*D. alata*	160	180	10	-	-	-	40	Hgaza et al. (2020)
Nigeria	*D. alata* *D. esculenta*	90^1 45^2 20^3	75^1 40^2 0^3	54^1 25^2 0^3	-	6	-	5–50	Frossard et al. (2017)
Pacific region	*D. alata* *D. rotundata* *D. esculenta*	30–76	26–78	0.7–8.7	0.5–3.3	1.0–4.5	1.5–2.7	15	O'Sullivan (2010)

1, 2, and 3 correspond to the nutrient recommendations on low, medium, and high soil fertility in Nigeria. An extrapolation is necessary if the potential yield is targeted. For 15 t ha^{-1}, O'Sullivan (2010) estimated requirements for oligo-elements at 10–14, 7.5–57, 21–270, 2.9–115, 36–95 g ha^{-1} for boron, copper, iron, manganese, and zinc, respectively, for the Pacific region

in West Africa. "Improving soil fertility (micronutrients, fertilizer, organic matter)" was listed as the second most important topic to be addressed in yam research after "improving shelf-life of yam tubers" by a global survey (Abdoulaye et al. 2014; Frossard et al. 2017). Integrated soil fertility management (ISFM), advocating the use of tolerant varieties with organic and chemical fertilizers, is promoted on yam (Frossard et al. 2017). However, its adoption is slow as farmers hardly afford external farm inputs and additional labor incurred by ISFM approaches. Besides, responses of this crop to fertilizers have been erratic and usually of low magnitude, compared with effects of other agronomic variables, such as staking or the size of planting setts (O'Sullivan 2010).

Breeding for more tolerant clones is then the most sustainable alternative for yam smallholder farmers. Apart from morphological screening and characterization of genotypes for low soil fertility tolerance (Diby et al. 2009; Dansi et al. 2013; Loko et al. 2015; Frossard et al. 2017; Matsumoto et al. 2021), no report exists on the use of conventional or biotechnological approaches for breeding low soil nutrient tolerant yam varieties. The need for screening available germplasm for tolerance to low soil fertility stems from the fact that genotypes have different sensitivity levels to extreme nutrient levels (O'Sullivan 2010; Matsumoto et al. 2021). They vary in their ability to take up nutrients when they are scarce or in their requirement for a particular nutrient to maintain their yield despite its scarcity. Genotypes also vary in their tolerance to toxic concentrations of mineral elements (O'Sullivan 2010). Using parental genotypes with tolerance to low soil fertility in crossing blocks, the development of progenies is ongoing under IITA yam breeding and is expect to open avenues for further genetic studies. It is noteworthy that field screening for nutrient deficiencies is often challenged by atypical symptoms compared with other crops. Since the benefits of long-fallowed land are tightly associated with soil microbiology, the research could be focused on changes in populations of mycorrhizal fungi, on which yams are highly dependent, and the ability of soil microorganisms to control populations of plant-parasitic nematodes (O'Sullivan 2010). Bio fertilizers could then be developed to mitigate the effects of short fallow due to intensive cropping. Regular organic manure application could result in similar results.

11.6 Conclusions and Prospects in Breeding Yam for Abiotic Stresses

Unlike breeding for disease resistance, abiotic stresses have not benefited much attention from yam breeders despite their adverse effects on tuber yield. Adverse effects of these stresses on root and tuber crops are expected to worsen with the climate change in West Africa which will mostly translate in increased temperatures and water shortages (McMahon 2013; Godfray 2014; Thiele et al. 2017). As the extensive use of external inputs (fertilizers, irrigation, etc.) is neither practical for resource-poor farmers nor ecologically friendly, plant breeders will need to provide

new cultivars better adapted to different farming systems and agro-ecologies under the context of climate change (Friedmann et al. 2018). With the expected double food demand in sub-Saharan Africa in 2050 due to exponential population growth, the increase in yield of staple food crops such as yam is urgent to ensure food and income security in the region (CGIAR 2016; Thiele et al. 2017; Friedmann et al. 2018). The conventional yam breeding will certainly not serve the cause since it takes at least 10 years to get a variety released. Besides, traditional approaches are limited by the low genetic variance of yield components under stress conditions, the complexity of abiotic stress tolerance traits, and the lack of efficient selection techniques (Nguyen et al. 2018).

Plants can overcome environmental stresses by activating molecular networks, including signal transduction, stress perception, metabolite production and expressions of specific stress-related genes (Nguyen et al. 2018).The approach of engineering plants to enhance abiotic stress resistance consists of strengthening these endogenous systems by intervening at different levels of the response, from signaling/regulatory elements and transcription factors to direct-action genes, effectors and antioxidant enzymes (Reguera et al. 2012; Nguyen et al. 2018). As for other crops, research in yam should investigate cellular responses in plants and discover regulatory and functional genes associated with abiotic stress tolerance. Discovery of genes will open avenues for MAS and transgenic and gene editing approaches to transfer these genes into plant genomes. Yam breeders should take advantage of the next-generation sequencing which is already used in yam research to facilitate the creation, by mutagenesis, of mutants involved in biosynthesis and roles of metabolites. As for biotic stress traits, technical advances should be explored to generate data set from genome-wide studies at the transcriptomic, proteomic, and metabolomic levels (Nguyen et al. 2018). The next step will be the conversion of identified QTLs to diagnostic markers. These markers will then go through verification and subsequent deployment in yam breeding programs. The application of these novel methods will enhance yam breeding efforts and ensure the quick delivery of high yielding, nutrient-dense and climate-resilient varieties to farmers in West Africa.

The effective incorporation of MAS in yam breeding will allow this crop to be efficiently and quickly improved by taking advantage of genomic advances reported in other crops such as maize, rice, potato, common beans, etc. for which the molecular research is sufficiently advanced. These advantages will include shortening the breeding cycle by speeding up the identification and transfer of desirable genes. Another key advantage of molecular markers in abiotic yam breeding will be to facilitate the pyramiding of genes from different sources of resistance for a more durable resistance to major stresses (Arnau et al. 2010).Use of biotechnological tools will also facilitate introgression of genes from secondary gene pools and wild relatives. Wild yam relatives such as *D. abyssinica*, *D. preahensilis*, *D. nummularia*, etc. are reputable sources for resistance and adaptation traits, and thus the use of biotechnology tools will facilitate introgression of resistance genes to popular yam species (Abraham et al. 2013; Adewumi et al. 2021; Mondo et al. 2021a).

In addition to incorporating biotechnology tools, effective breeding for abiotic stresses (such as flooding, heat, drought, salinity, etc.) will require well-refined protocols for field and greenhouse phenotyping. Most of these traits are complex; understanding of their physiological and genetic bases is still incomplete even for model crops, making specific genetic targets rare (Ishitani et al. 2011). Protocols already used for other crops can be adapted in the context of yam (see Polania et al. 2012a, b; Thiele et al. 2017).

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References

Abdoulaye T, Alene A, Rusike J, Adebayo A (2014) Results of a global online expert survey: major constraints, opportunities, and trends for yam production and marketing and priorities for future RTB yam research. In: CGIAR research program on Roots, Tubers and Bananas (RTB), RTB working paper 2014-3. Available online www.rtb.cgiar.org

Abraham K (1992) Dormancy and viability of *Dioscoreaalata* seeds. J Root Crops 18:142–143

Abraham K, Nair PG (1990) Floral biology and artificial pollination in *Dioscoreaalata* L. Euphytica 48(1):45–51

Abraham K, Nair PG (1991) Polyploidy and sterility in relation to sex in *Dioscoreaalata* L. (Dioscoreaceae). Genetica 83(2):93–97.

Abraham K, Nair SG, Sreekumari MT, Unnikrishnan M (1986) Seed set and seedling variation in greater yam (*Dioscoreaalata* L.). Euphytica 35(2):337–343.

Abraham K, Némorin A, Lebot V, Arnau G (2013) Meiosis and sexual fertility of autotetraploid clones of greater yam *Dioscorea alata* L. Genet Resour Crop Evol 60(3):819–823

Adeniji MO, Shoyinka SA, Ikotun T, Asiedu R, Hughes Jd'A et al (2012) Yield loss in Guinea yam (*Dioscorea rotundata* Poir.) due to infection by Yam mosaic virus (YMV) Genus Potivirus. Ife J Sci 14(2):237–244

Adewumi AS, Asare PA, Adu MO, Taah KJ et al (2021) Farmers' perceptions on varietal diversity, trait preferences and diversity management of bush yam (*Dioscoreapraehensilis* Benth.) in Ghana. Sci Afr 12:e00808

Adifon FH, Yabi I, Vissoh P, Balogoun I, Dossou J et al (2019) Écologie, systèmes de culture et utilisations alimentaires des ignames en Afrique tropicale: synthèse bibliographique. Cah Agri 28:22

Agre P, Asibe F, Darkwa K, Edemodu A, Bauchet G et al (2019) Phenotypic and molecular assessment of genetic structure and diversity in a panel of winged yam (*Dioscorea alata*) clones and cultivars. Sci Rep 9(1):1–11

Agre P, Nwachukwu C, Olasanmi B, Obidiegwu J, Nwachukwu E et al (2020) Sample preservation and plant sex prediction in white Guinea yam (*Dioscorearotundata* Poir.). J Appl Biotechnol Rep 7(3):145–151.

Agre P, Norman PE, Asiedu R, Asfaw A (2021) Identification of quantitative trait nucleotides and candidate genes for tuber yield and mosaic virus tolerance in an elite population of white Guinea yam (*Dioscorea rotundata*) using genome-wide association scan. BMC Plant Biol. https://doi.org/10.21203/rs.3.rs-612999/v1

Aighewi BA, Asiedu R, Maroya N, Balogun M (2015) Improved propagation methods to raise the productivity of yam (*Dioscorearotundata* Poir.). Food Secur 7:823–834

Akakpo R, Scarcelli N, Chaïr H, Dansi A, Djedatin G et al (2017) Molecular basis of African yam domestication: analyses of selection point to root development, starch biosynthesis, and photosynthesis related genes. BMC Genomics 18:782–791

Alabi TR, Adebola PO, Asfaw A, De Koeyer D, Lopez-Montes A et al (2019) Spatial multivariate cluster analysis for defining target population of environments in West Africa for yam breeding. Intl J Appl Geospat Res 10(3):1–30

Arnau G, Abraham K, Sheela MN, Chair H, Sartie A, Asiedu R (2010) Yams. In: Bradshaw JE (ed) Root and tuber crops: hand book of plant breeding, 7th edn. Springer, New York, USA, pp 127–148

Arnau G, Némorin A, Maledon E, Abraham K (2009) Revision of ploidy status of *Dioscorea alata* L. (Dioscoreaceae) by cytogenetic and microsatellite segregation analysis. Theor Appl Genet 118(7):1239–1249

Asemota HN, Ramser J, Lopez-Peralta C, Weising K, Kahl G (1995) Genetic variation and cultivar identification of Jamaican yam germplasm by random amplified polymorphic DNA analysis. Euphytica 3:341–351

Asfaw A, Aderonmu DS, Darkwa K, De Koeyer D, Agre P et al (2021) Genetic parameters, prediction, and selection in a white Guinea yam early-generation breeding population using pedigree information. Crop Sci 61:1038–1051

Atu UG, Odurukwe SO, Ogbuji RO (1983) Root-knot nematode damage to *Dioscorea rotundata.* Plant Dis 67(7):814–815

Azeteh IN, Hanna R, Sakwe PN, Njukeng AP, Kumar PL (2019) Yam (*Dioscorea* spp.) production trends in Cameroon: a review. Afr J Agri Res1 4(26):1097–1110

Babil P, Iino M, Kashihara Y, Matsumoto R, Kikuno H et al (2016) Somatic polyploidization and characterization of induced polyploids of *Dioscorea rotundata* and *Dioscorea cayenensis.* Afr J Biotechnol 15(38):2098–2105

Bai KV, Ekanayake IJ (1998) Taxonomy, morphology and floral biology. In: Orkwor GC, Asiedu R, Ekanayake IJ (eds) Food yams: advances in research. NRCRI and IITA, Ibadan, Nigeria, pp 13–38

Bhattacharjee R, Nwadili CO, Saski CA, Paterne A, Scheffler BE et al (2018) An EST-SSR based genetic linkage map and identification of QTLs for anthracnose disease resistance in water yam (*Dioscorea alata* L.). PLoS One 13(10):e0197717. https://doi.org/10.1371/journal.pone.0197717

Bhattacharjee R, Agre P, Bauchet G, De Koeyer D, Lopez-Montes A et al (2020) Genotyping-by-sequencing to unlock genetic diversity and population structure in white yam (*Dioscorea rotundata* Poir.). Agronomy 10(9):1437

Bino RJ, Hall RD, Fiehn O, Kopka J, Saito K et al (2004) Potential of metabolomics as a functional genomics tool. Trends Plant Sci 9(9):418–425

Bonire JJ (1985) Preventing yam rot with organotin compounds. Niger J Sci 19:145–148

Bousalem M, Arnau G, Hochu I, Arnolin R, Viader V et al (2006) Microsatellite segregation analysis and cytogenetic evidence for tetrasomic inheritance in the American yam *Dioscorea trifida* and a new basic chromosome number in the Dioscorea. Theor Appl Genet 113(3):439–451

Bradshaw JE (2017) Plant breeding: past, present and future. Euphytica 213(3):60

Bredeson JV, Lyons JB, Oniyinde IO, Okereke NR, Kolade O et al (2021) Chromosome evolution and the genetic basis of agronomically important traits in greater yam. BioRxiv. https://doi.org/10.1101/2021.04.14.439117v1.abstract

CGIAR Research Program on Roots, Tubers and Bananas (RTB) (2016) Roots, Tubers and Bananas (RTB) Proposal 2017–2022. https://cgspace.cgiar.org/handle/10947/4286. Accessed 6th January 2020

Chen X, Cai L, Zhang Y, Su W, Li B et al (2020) The complete chloroplast genome sequence of the *Dioscorea esculenta* (Lour.) Burkill (Dioscoreaceae). Mitochon DNA Part B 5(3): 3786–3788
Cormier F, Lawac F, Maledon E, Gravillon MC, Nudol E et al (2019) A reference high-density genetic map of greater yam (*Dioscoreaalata* L.). Theor Appl Genet 132(6):1733–1744
Cormier F, Martin G, Vignes H, Lachman L, Cornet D et al (2021) Genetic control of flowering in greater yam (*Dioscoreaalata* L.). BMC Plant Biol 21(1):1–12
Cornet D(2005) Etude du fonctionnement physiologique d'un couvert végétal d'igname (*Dioscoreaalata* L.). Doctoral dissertation, Université de Gembloux, Belgium
Dansi A, Dantsey-Barry H, Dossou-Aminon I, Kpenu EK, Agré AP et al (2013) Varietal diversity and genetic erosion of cultivated yams (*Dioscoreacayenensis* Poir-*D. rotundata* Lam complex and *D. alata* L.) in Togo. Intl J Biodivers Conserv 5(4):223–239
Dare MO, Fagbola O, Abaidoo RC, Asiedu R (2014) Evaluation of white yam (*Dioscorea rotundata*) genotypes for arbuscular mycorrhizal colonization, leaf nutrient concentrations and tuber yield under NPK fertilizer application. J Plant Nutri 37(5):658–673
Darkwa K, Agre P, Olasanmi B, Iseki K, Matsumoto R et al (2020a) Comparative assessment of genetic diversity matrices and clustering methods in white Guinea yam (*Dioscorea rotundata*) based on morphological and molecular markers. Sci Rep 10(1):1–14
Darkwa K, Olasanmi B, Asiedu R, Asfaw A (2020b) Review of empirical and emerging breeding methods and tools for yam (*Dioscorea* spp.) improvement: status and prospects. Plant Breed 139(3):474–497
Defrance D, Sultan B, CastetsM FAM, Baron C (2020) Impact of climate change in West Africa on cereal production per capita in 2050. Sustainability 12(18):7585
Degras LM (1969) Quelques donnees sur la variabilite de descendances d'igname cousse-couche (*D. trifida* L.). In: Proceedings, seventh annual meeting. Caribean Food Crops Society, Martinique, Guadeloupe
Diby LNG, Hgaza VK, Tie TB, Assa A, Carsky R et al (2009) Productivity of yams (*Dioscorea* spp.) as affected by soil fertility. J Anim Plant Sci 5(2):494–506
Diby LN, Hgaza VK, Tié TB, Assa A, Carsky R et al (2011) How does soil fertility affect yam growth? Acta Agri Scand Sect BSoil Plant Sci 61(5):448–457
Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K et al (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE 6(5):e19379
FAO (2020) FAO stat. http://www.fao.org/faostat/en/#data/QC
Feng S, Song W, Fu R, Zhang H, Xu A et al (2018) Application of the CRISPR/Cas9 system in *Dioscoreazingiberensis*. Plant Cell Tiss Org Cult 135(1):133–141
Fernie AR, Schauer N (2009) Metabolomics-assisted breeding: a viable option for crop improvement? Trends Genet 25(1):39–48
Floquet AB, Maliki R, Tossou RC, Tokpa C (2012) Évolution des systèmes de production de l'igname dans la zone soudano-guinéenne du Bénin. Cah Agri 21:427–437
Friedmann M, Asfaw A, Anglin NL, Becerra LA, Bhattacharjee R et al (2018) Genomics-assisted breeding in the CGIAR research program on Roots, Tubers and Bananas (RTB). Agriculture 8:89
Friedmann M, Becerra LA, Fraser PD (2019) Metabolomics in the CGIAR Research Program on Roots, Tubers and Bananas (RTB). In: Lima (Peru). CGIAR research program on Roots, Tubers and Bananas (RTB). RTB working paper. No. 2019-2. www.rtb.cgiar.org
Frossard E, Aighewi BA, Aké S, Barjolle D, Baumann P et al (2017) The challenge of improving soil fertility in yam cropping systems of West Africa. Front Plant Sci 8:1953
Gao R, Shi X, Ma Z, Li Y, Wen J (2018) Physiological responses to drought in three provenances of *Discoreanipponica* Makino. J Appl Bot Food Qual 91:261–270
Gatarira C, Agre P, Matsumoto R, Edemodu A, Adetimirin V et al (2020) Genome-wide association analysis for tuber dry matter and oxidative browning in water yam (*Dioscoreaalata* L.). Plants 9(8):969
Gatarira C, Sládeková L, Němečková A, Šimoníková D, Paliwal R et al (2021) Cytological and molecular characterization for ploidy determination in yams (*Dioscorea* spp.). Agronomy 11(10):1897

Girma G, Hyma KE, Asiedu R, Mitchell SE, Gedil M et al (2014) Next-generation sequencing based genotyping, cytometry and phenotyping for understanding diversity and evolution of Guinea yams. Theor Appl Genet 127(8):1783–1794

Girma G, Gedil M, Spillane C (2015) Morphological, SSR and ploidy analysis of water yam (*Dioscorea alata* L.) accessions for utilization of aerial tubers as planting materials. Genet Resour Crop Evol 2:291–305

Girma G, Natsume S, Carluccio AV, Takagi H, Matsumura H et al (2019) Identification of candidate flowering and sex genes in white Guinea yam (*D. rotundata* Poir.) by Super SAGE transcriptome profiling. PLoS One 14 (9):e0216912

Godfray HCJ (2014) How can 9–10 billion people be fed sustainably and equitably by 2050? In: Goldin I (ed) Is the planet full? Oxford University Press, Oxford, pp 104–120

Govaerts R, Wilkin P, Saunders RMK (2007) World checklist of dioscoreales; yams and their allies: the board of trustees of the royal botanic gardens. Kew, London, UK, pp 1–65

Govaerts R, Wilkin P, Raz L,Téllez-Valdés O (2017) World checklist of dioscoreaceae. Royal Botanic gardens, Kew. http://apps.kew.org/wcsp/. Accessed 1 June 2021

Hgaza VK, Oberson A, Kiba DI, Diby LN, Aké S et al (2020) The nitrogen nutrition of yam (*Dioscorea* spp). J Plant Nutr 43:64–78

Igwilo N, Udeh AC (1987) Effect of waterlogging yam vines on vegetative growth and tuber yield. Ann Appl Biol 110(2):391–398

IITA (2018) Our genetic resources. Retrieved from http://www.iita.org/research/genetic-resources/

Ijato J, Tedela P (2015) Phytotoxic potentials of cold and hot aqueous extracts of *Chromolaena odorata* against fungal deteriorating agents of yam tubers (*Dioscorearotundata*, Poir) after harvest. Amer J Exp Agri 5:262–266

Ishitani M, Rane J, Beebe S, Sankaran M, Blair M et al (2011) Molecular breeding approach in managing abiotic stresses. Biol Breed Food Legumes 276

Jarvis A, Ramirez-Villegas J, Herrera Campo BV, Navarro-Racines C (2012) Is cassava the answer to African climate change adaptation? Trop Plant Biol 5(1):9–29

Kolombia YA, Karssen G, Viaene N, Kumar PL, De Sutter N et al (2017) Diversity of root-knot nematodes associated with tubers of yam (*Dioscorea* spp.) established using isozyme analysis and mitochondrial DNA-based identification. J Nematol 49(2):177

Kouakou AM, Yao GF, Dibi KEB, Mahyao A, Lopez-Montes A et al (2019) Yam cropping system in Cote d'Ivoire: current practices and constraints. Eur Sci J 15:30

Lebot V, Abraham K, Kaoh J, Rogers C, Molisalé T (2019a) Development of anthracnose resistant hybrids of the greater Yam (*Dioscorea alata* L.) and interspecific hybrids with *D. nummularia* Lam. Genet Resour Crop Evol 66:871–883

Lebot V, Malapa R, Molisalé T (2019b) Development of HP-TLC method for rapid quantification of sugars, catechins, phenolic acids and saponins to assess Yam (*Dioscorea* spp.) tuber flour quality. Plant Genet Resour Charact Utiliz 17(1):62–72

Liu L, Huang Y, Huang X, Yang J, Wu W et al (2017) Characterization of the dioscorin gene family in *Dioscorea alata* reveals a role in tuber development and environmental response. Intl J Mol Sci 18(7):1579

Loko YL, Adjatin A, Dansi A, Vodouhè R, Sanni A (2015) Participatory evaluation of Guinea yam (*Dioscorea cayenensis* Lam.–*D. rotundata* Poir. complex) landraces from Benin and agro-morphological characterization of cultivars tolerant to drought, high soil moisture and chips storage insects. Genet Resour Crop Evol 62(8):1181–1192

Loko YL, Bhattacharjee R, Agre AP, Dossou-Aminon I, Orobiyi A et al (2017) Genetic diversity and relationship of Guinea yam (*Dioscorea cayenensis* Lam.–*D. rotundata* Poir. complex) germplasm in Benin (West Africa) using microsatellite markers. Genet Resour Crop Evol 64(6):1205–1219

McMahon P (2013) Feeding frenzy. Profile Books, London

Manoharan R, Tripathi JN, Tripathi L (2016) Plant regeneration from axillary bud derived callus in white yam (*Dioscorea rotundata*). Plant Cell Tiss Org Cult 126(3):481–497

Matsumoto R, Ishikawa H, Asfaw A, Asiedu R (2021) Low soil nutrient tolerance and mineral fertilizer response in white Guinea yam (*Dioscorea rotundata*) genotypes. Front Plant Sci 12:223

McKoy ML, Thomas PG, Asemota H, Omoruyi F,Simon O (2014) Effects of Jamaican bitter yam (*Dioscoreapolygonoides*) and diosgenin on blood and fecal cholesterol in rats. J Med Food 17:1183–1188

Mignouna DB, Akinola AA, Abdoulaye T, Alene AD, Manyong V et al (2020) Potential returns to yam research investment in sub-Saharan Africa and beyond. Outlook Agri 49(3):215–224

Mignouna HD, Ellis NTH, Knox MR, Asiedu R, Ng QN (1998) Analysis of genetic diversity in Guinea yams (*Dioscorea* spp.) using AFLP fingerprinting. Trop Agri 75:224–229

Mignouna H, Mank R, Ellis T, Van Den Bosch N, Asiedu R et al (2002a) A genetic linkage map of Guinea yam (*Dioscorearotundata* Poir.) based on AFLP markers. Theor Appl Genet 105(5):716–725

Mignouna H, Mank R, Ellis T, Van Den Bosch N, Asiedu R et al (2002b) A genetic linkage map of water yam (*Dioscoreaalata* L.) based on AFLP markers and QTL analysis for anthracnose resistance. Theor Appl Genet1 05(5):726–735

Mondo JM, Agre PA, Edemodu A, Adebola P, Asiedu R et al (2020) Floral biology and pollination efficiency in yam (*Dioscorea* spp.). Agriculture 10(11):560

Mondo JM, Agre PA, Edemodu A, Asiedu R, Akoroda MO et al (2021a) Cross-compatibility analysis in intra-and interspecific breeding relationships in yam (*Dioscorea* spp.). Sci Rep. In press

Mondo JM, Agre PA, Asiedu R, Akoroda MO, Asfaw A (2021b) Genome-wide association studies for sex determination and cross-compatibility in water yam (*Dioscoreaalata* L.). Plants 10:1412

Mondo JM, Agre PA, Asiedu R, Akoroda MO, Asfaw A (2021c) Optimized protocol for in vitro pollen germination in yam (*Dioscorea spp.*). Plants 10(4):795

Mulualem T, Mekbib F, Shimelis H, Gebre E, Amelework B (2018) Genetic diversity of yam (*Dioscorea* spp.) landrace collections from Ethiopia using simple sequence repeat markers. Aust J Crop Sci 12(8):1222–1230

Narina SS, Buyyarapu R, Kottapalli KR, Sartie AM, Ali MI et al (2011) Generation and analysis of expressed sequence tags (ESTs) for marker development in yam (*Dioscoreaalata* L.). BMC Genomics 12(1):1–10

Neina D (2021) Ecological and edaphic drivers of yam production in West Africa. Appl Environ Soil Sci 5019481

Nguyen HC, Lin KH, Ho SL, Chiang CM, Yang CM (2018) Enhancing the abiotic stress tolerance of plants: from chemical treatment to biotechnological approaches. Physiol Planta 164(4):452–466

Nkhoma N, Shimelis H, Laing MD, Shayanowako, A, Mathew I (2020) Assessing the genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] germplasm collections using phenotypic traits and SNP markers. BMC Genet 21:110–126

Norman PE, Agre AP, Danquah A, Tongoona PB, Danquah EY et al (2020) Paternity assignment in white Guinea yam (*Dioscorea rotundata*) half-sib progenies from polycross mating design using SNP markers. Plants 9:527

Norman PE, Asfaw A, Tongoona PB, Danquah A, Danquah EY et al (2018) Pollination success in some white yam genotypes under polycross and nested mating designs. Intl J Biol Sci Appl 5:19–28

Norman PE, Danquah A, Asfaw A, Tongoona PB, Danquah EY et al (2021) Seed viability, seedling growth and yield in white Guinea yam. Agronomy 11(2). https://doi.org/10.3390/agronomy11010002

Nyaboga E, Tripathi JN, Manoharan R, Tripathi L (2014) Agrobacterium-mediated genetic transformation of yam (*Dioscorea rotundata*): an important tool for functional study of genes and crop improvement. Front Plant Sci 5:463

Odoh NC (2014) Drought tolerance variation among yam genotypes and the contributory role of arbuscular mycorrhizal fungi. PhD thesis department of agronomy, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria

Omohimi C, Piccirillo C, Roriz M, Ferraro V, Vasconcelos WM et al (2017) Study of the proximate and mineral composition of different Nigerian yam chips, flakes and flours. J Food Sci Technol 55:42–51

Onwueme IC (1975) Tuber formation in yam (*Dioscorea* spp.): effect of moisture stress; contribution of the parent sett. J Agri Sci 85(2):267–269

Onwueme IC, Charles WB (1994) Tropical root and tuber crops: production, perspectives and future prospects (No. 126). Food & Agriculture Organization, Rome, Italy

O'Sullivan JN (2010) Yam nutrition: nutrient disorders and soil fertility management. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia, p 112

Otoo E (2017) Yam breeding in Ghana. J Agri Sci 9:122

Ousmael KM, Tesfaye K, Hailesilassie T (2019) Genetic diversity assessment of yams (*Dioscorea* spp.) from Ethiopia using inter simple sequence repeat (ISSR) markers. Afr J Biotechnol 18(30):970–977

Penet L, Barthe E, Alleyne A, Blazy JM (2016) Disease risk perception and diversity of management strategies by farmers: The case of anthracnose caused by *Colletotrichum gloeosporioides* on water yams (*Dioscorea alata*) in Guadeloupe. Crop Protec 88:7–17

Polanía JA, RiveraM, Ricaurte J, Rao IM (2012a) Phenotyping common beans for adaptation to drought: protocol for greenhouse evaluation. In: International Center for Tropical Agriculture (CIAT), Cali, Colombia, 13 pp.

Polanía JA, Rivera M, Rao IM, Ricaurte J (2012b) Phenotyping common beans for adaptation to drought: protocol for field evaluation. In: International Center for Tropical Agriculture (CIAT), Cali, Colombia, 19 pp.

Pretty J, Toulmin C, Williams S (2011) Sustainable intensification in African agriculture. Intl J Agri Sustain 9:5–24

Price EJ, Bhattacharjee R, Lopez-Montes A, Fraser PD (2017) Metabolite profiling of yam (*Dioscorea* spp.) accessions for use in crop improvement programs. Metabolomics 13(11):144

Price EJ, Bhattacharjee R, Lopez-Montes A, Fraser PD (2018) Carotenoid profiling of yams: clarity, comparisons and diversity. Food Chem 259:130–138

Price EJ, Drapal M, Perez-Fons L, Amah D, Bhattacharjee R et al (2020) Metabolite database for root, tuber, and banana crops to facilitate modern breeding in understudied crops. Plant J 101(6):1258–1268

Price EJ, Wilkin P, SarasanV FPD (2016) Metabolite profiling of *Dioscorea* (yam) species reveals under-utilized biodiversity and renewable sources for high-value compounds. Sci Rep 6:29136

RegueraM PZ, Blumwald E (2012) Targeting metabolic pathways for genetic engineering abiotic stress-tolerance in crops. Biochim Biophys Acta 1819(2):186–194

Remesh KR, Byju G, Soman S, Raju S, Ravi V (2019) Future changes in mean temperature and total precipitation and climate suitability of yam (*Dioscorea* spp.) in major yam-growing environments in India. CurrHort 7(1):28–42

Sadik S, Okereke OU (1975) A new approach to improvement of yam *Dioscorea rotundata.* Nature 254(5496):134–135

Saski CA, Bhattacharjee R, Scheffler BE, Asiedu R (2015) Genomic resources for water yam (*Dioscoreaalata* L.): Analyses of EST-sequences, *de novo* sequencing and GBS libraries. PLoS One 10(7):e0134031

Scarcelli N, Cubry P, Akakpo R, Thuillet A-C, Obidiegwu J et al (2019) Yam genomics supports West Africa as a major cradle of crop domestication. SciAdv 5(5):eaaw1947

Scarcelli N, Tostain S, Vigouroux Y, Agbangla C, Dainou O et al (2006) Farmers' use of wild relative and sexual reproduction in a vegetatively propagated crop. The case of yam in Benin. Mol Ecol 15:2421–2431

Siadjeu C, Mayland-Quellhorst E, Albach DC (2018) Genetic diversity and population structure of trifoliate yam (*Dioscorea dumetorum* Kunth) in Cameroon revealed by genotyping-by-sequencing (GBS). BMC Plant Biol 18(1):1–14

Siadjeu C, Pucker B, Viehöver P, Albach DC, Weisshaar B (2020) High contiguity *de novo* genome sequence assembly of Trifoliate yam (*Dioscorea dumetorum*) using long read sequencing. Genes 11(3):274

Siadjeu C, Mayland-Quellhorst E, Pande S, Laubinger S, Albach DC (2021) Transcriptome sequence reveals candidate genes involving in the post-harvest hardening of trifoliate yam *Dioscorea dumetorum*. Plants 10(4):787

Spindel J, Wright M, Chen C, Cobb J, Gage J et al (2013) Bridging the genotyping gap: using genotyping by sequencing (GBS) to add high-density SNP markers and new value to traditional bi-parental mapping and breeding populations. Theor Appl Genet 126(11):2699–2716

Srivastava AK, Gaiser T, Paeth H, Ewert F (2012) The impact of climate change on yam (*Dioscorea alata*) yield in the savanna zone of West Africa. Agri Ecosyst Environ 153:57–64

Stanley A, Menkir A, Agre P, Ifie B, Tongoona P et al. (2020) Genetic diversity and population structure of maize inbred lines with varying levels of resistance to *Striga hermonthica* using agronomic trait-based and SNP markers. Plants 9:1223–1239

Sugihara Y, Darkwa K, Yaegashi H, Natsume S, Shimizu M et al (2020) Genome analyses reveal the hybrid origin of the staple crop white Guinea yam (*Dioscorea rotundata*). Proc Natl Acad SciUSA 117(50):31987–31992

Sugihara Y, Kudoh A, Oli MT, Takagi H, Natsume S et al (2021) Population genomics of yams: evolution and domestication of dioscorea species. In: Population genomics. Springer, Cham

Syombua ED, Zhang Z, Tripathi JN, Ntui VO, Kang M et al (2021) A CRISPR/Cas9-based genome-editing system for yam (*Dioscorea* spp.). Plant Biotechnol J19(4):645–647

Tamiru M, Natsume S, Takagi H, White B, Yaegashi H et al (2017) Genome sequencing of the staple food crop white Guinea yam enables the development of a molecular marker for sex determination. BMC Biol 15:1–20

Tamiru M, Yamanaka S, Mitsuoka C, Babil P, Takagi H et al (2015) Development of genomic simple sequence repeat markers for yam. Crop Sci 55:2191–2200

Terauchi R, Kahl G (1999) Mapping of the *Dioscoreatokoro* genome: AFLP markers linked to sex. Genome 42(4):752–762

Thiele G, Khan A, Heider B, Kroschel J, Harahagazwe D et al (2017) Roots, tubers and bananas: planning and research for climate resilience. Open Agri 2(1):350–361

Thottathil GP, Jayasekaran K, Othman AS (2016) Sequencing crop genomes: a gateway to improve tropical agriculture. Tropical Life Sci Res 27(1):93

Wilkin P, Schols P, Chase MW, Chayamarit K, Furness CA et al (2005) A plastid gene phylogeny of the yam genus, *Dioscorea*: roots, fruits and Madagascar. Syst Bot 30(4):736–749

Wu ZG, Jiang W, Mantri N, Bao XQ, Chen SL et al (2015) Transciptome analysis reveals flavonoid biosynthesis regulation and simple sequence repeats in yam (*Dioscoreaalata* L.) tubers. BMC Genomics 16(1):346

Zhao-wei LI (2012) Studies on callus tissue culture for different explants of *Dioscorea opposita*. J Anhui Agri Sci 25

Zhu Q, Wu F, Ding F, Ye D, Chen Y, Li Y, Zhifan Y (2009) Agrobacterium-mediated transformation of *Dioscorea zingiberensis* Wright, an important pharmaceutical crop. Plant Cell Tiss Org Cult 96(3):317–324