Chittaranjan Kole *Editor*

Genomic Designing for Abiotic Stress Resistant Vegetable Crops

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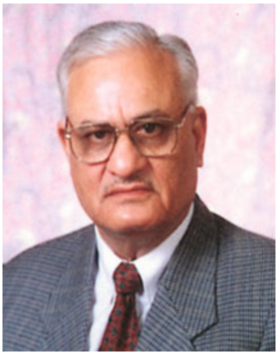

Dr. K. L. Chadha

Padma Shri Awardee, former Deputy Director General (Horticulture), Indian Council of Agricultural Research and Founder President of the Indian Academy of Horticultural Sciences

With regards and 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 10–15% 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 has 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 F^5 utilities; nutritional and neutraceutical compounds; and other industrially and aesthetically important products, and possibility of multiple utilities. For this purpose of 'precise' breeding employment of the genetic and genomic techniques individually or in combination as and when required, will play a crucial role.

The chapters of the 12 volumes of this twin book series entitled, "Genomic Designing for Biotic Stress Resistant Crops" and "Genomic Designing for Abiotic Stress Resistant Crops", will deliberate on different types of biotic and abiotic stresses and their effects on and interaction with crop plants; will enumerate the available genetic diversity with regard to biotic or abiotic stress resistance among cultivars; 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 Vegetable Crops” includes nine chapters focused on tomato, potato, Capsicums, eggplant, vegetable Brassicas, cucurbits, onion and garlic, amaranth and carrot contributed by 51 scientists from 6 countries including Greece, India, Italy, Israel, Malaysia and UK. 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

ABA	Abscisic acid
ABRE	ABA-responsive element
ACC	1-aminocyclopropane-1-carboxylic acid
ADC	Arginine decarboxylase
ADH	Alcohol dehydrogenase
AFLP	Amplified fragment length polymorphism
AFP	Antifreeze protein
Am	Association mapping
AMF	Arbuscular mycorrhizal fungi
AMOVA	Analysis of marker variance
Anx D1	Annexin D1
AOX	Alternative oxidase
APX	Ascorbate peroxidase
AQP	Aquaporin
AS	Alternate splicing
AtCBF3	*A. thaliana* C-repeat binding factor 3
AtCOR15A	*A. thaliana* cold-regulated 15A
AtRD29A	*A. thaliana* Responsive To Desiccation 29A
BAC	Bacterial artificial chromosome
BC1	First backcross generation
BC2	Second backcross generation
BIM	Bayesian interval mapping
BLAST	Basic local alignment search tool
BLUP	Best linear unbiased prediction
BR	*Brassica/Raphanus*
BSA	Bulked segregant analysis
BSR-Seq	BSA RNA-Seq
C4H	Cinnamate-4-hydroxylase
C5-MTases	Cytosine-5-methyltransferases
CAPS	Cleaved amplified polymorphic sequence
Cas9	CRISPR-associated protein 9

CAT	Catalase
CC	Core collection
CCC	Colombian central collections
CComT	Caffeoyl-CoA O-methyltransferase
cDNA	Complementary DNA
CDS	Coding sequence
CE-MS	Capillary electrophoresis-mass spectrometry
CFRB	Coordinated framework for regulation of biotechnology
CGIAR	Consultative Group on International Agricultural Research
CI	Chilling injury
CIM	Composite interval mapping
CIP	International Potato Center
CMS	Cytoplasmic male sterility
CNV	Copy number variation/variant
COMT	Caffeic acid-O-methyltransferase
COS	Conserved ortholog set
CPC	Commonwealth Potato Collection
CPR	Constitutive expression of pathogenesis-related gene
CR	Cold responsive
CRISPR	Clustered regularly interspaced short palindromic repeats
CRT	C-repeat
CS	Capsaicin synthase
CWR	Crop wild relative
DArT	Diversity array technology
DDBJ	DNA Data Bank in Japan
DEG	Differentially expressed gene
DH	Doubled haploid
DM	Doubled monoploid
DOF	DNA-binding with one finger gene family
DOT	Dihydroxyphenylalanine oxidation tyrosinase
DRE	Dehydration-responsive element
DREB	Dehydration-responsive element binding factor
DTE	Drought-tolerant efficiency
DUS	Distinctness, uniformity and stability
EBN	Endosperm balance number
EBVs	Estimated breeding values
ECPD	European Cultivated Potato Database
ECPGR	European Cooperative Programme for Plant Genetic Resources
EDPG	Extracellular dermal glycoprotein
EMBL	European Molecular Biology Laboratory
EMBO	European Molecular Biology Organization
EMS	Ethylmethane sulfanate
ERF	Ethylene response factor
EST	Expressed sequence tag
ET	Evapotranspiration

EUG	Eugenol
FAFLP	Fluorescent amplified fragment length polymorphism
FAO	Food and Agricultural Organizations
FAOSTAT	FAO Corporate Statistical Database
FBA	F-box associated
FBA	Fructose 1, 6 bisphosphate aldolase
FC	Field capacity
FISH	Fluorescence in situ hybridization
FLC	*Flowering locus C*
FLD	*Flowering locus D*
GA	Gibberellic acid
GABA	Gamma amino butyric acid
GB	Glycine betaine
GBS	Genotyping-by-sequencing
GC-MS	Gas chromatography-mass spectrometry
GE	Genome editing
GEBV	Genomic estimated breeding value
GFP	Green fluorescent protein
GM	Genetically modified
GMO	Genetically modified organism
GO	Gene ontology
GR	Glutathione reductase
GS	Genomic selection
GSH	Ascorbate–glutathione
GST	Glutathione S-transferase
GUS	β-Glucuronidase
GWAS	Genome-wide association study/studies
GxE	Genotype x environment interaction
HD-Zip	Homeodomain leucine zipper protein
HI-C	High chromosome conformation capture
HKT	High-affinity potassium transporter
HPLC	High-performance liquid chromatography
HRM	High-resolution melting
HSF	Heat shock factor
Hsp/HSP	Heat shock protein
HT	Heat tolerance
HTG	High-throughput genotyping
HTHH	High temperature and high humidity stress
HTP	High-throughput phenotyping
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IL	Introgression line
InDel	Insertion/deletion
IPCC	Intergovernmental Panel on Climate Change
IPT	Isopentyl transferase
ISSR	Inter-simple sequence repeat

ITAG	International Tomato Annotation Group
iTRAQ	Isobaric tag for relative and absolute quantification.
KAS	Keto acyl synthase
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS	Liquid chromatography-mass spectrometry
LD	Linkage disequilibrium
LEA	Late embryogenesis abundant
LOD	Logarithm of odds
LRR	Leucine-rich repeat
LTC	Low-temperature conditioning
MAGIC	Multiparental advanced generation intercross
MAPK	Mitogen-activated protein kinase
MAS	Marker-assisted selection
MBT	Mother and baby trial
MDA	Malondialdehyde
MeJA	Methyl jasmonate
MeSA	Methyl salicylate
MIM	Multiple interval mapping
MIP	Major intrinsic protein
miRNA	MicroRNA
MLO	*Mildew resistance locus*
MOD	Model Organism Database
MQTL	Meta-QTL
MSI	Membrane stability index
mtlD	Mannitol-1-phosphodehydrogenase
NAD-ME	NAD-dependent malic enzyme
NADP	Nicotinamide adenine dinucleotide phosphate
NADP-ME	NADP-dependent malic enzyme
NAM	Nested association mapping
NBS	Nucleotide-binding site
NCBI	National Center for Biotechnology Information
NDPK	Nucleoside diphosphate kinase
NEU	*N*-nitroso-*N*-ethylurea
NGS	Next-generation sequencing
NIL	Near isogenic line
NMR	Nuclear magnetic resonance
NMU	*N*-Nitroso-*N*-methylurea
NPBT	New plant breeding techniques
NPTII	Neomycin phosphotransferase II
Nr	Never ripe
NUE	Nutrient-use efficiency
OA	Osmotic adjustment
OE	Overexpression lines
ORC5	Origin recognition complex subunit 5
ORF	Open reading frame

OSM	Osmotin protein
PAGE	Polyacrylamide gel electrophoresis
PAL	Phenyl ammonia lyase
pAMT	Putative aminotransferase
PAV	Presence/absence variants/variations
PCR	Polymerase chain reaction
PD	Protein disulfide isomerase
PEG	Poly ethylene glycol
PEPCK	PEP carboxykinase
PGD	Pepper Genome Database
PGDB	Pathway/Genome Databases
PGIP	Polygalacturonase inhibitor protein
PGSC	Potato Genome Sequencing Consortium
PLD	Phospholipase D
Pn	Photosynthesis
POD	Peroxidase
POLD1	DNA polymerase delta1
PPO	Polyphenol oxidase
PPV&FR	Protection of Plant Varieties and Farmers' Rights
pQTL	Plasticity QTL
PR	Pathogenesis-related
PR1	Pathogenesis-related Protein 1
PS II	Photosystem II
Q	Ubiquinone
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
RAD	Restriction site-associated DNA
Rad-Seq	Restriction site-associated DNA sequencing
RAPD	Random amplified polymorphic DNA
RBS	Ribosome-binding site
RDI	Regulated deficit irrigation
REL	Relative electrolyte leakage
REMAP	Retrotransposon-microsatellite amplified polymorphism
RFLP	Restriction fragment length polymorphism
RFO	Raffinose family oligosaccharide
RGA	Resistance gene analog
R-gene	Resistance gene
RIL	Recombinant inbred line
RLK	Receptor-like kinase
RLP	Receptor-like protein
RNAi	RNA interference
RNA-seq	RNA sequencing
ROS	Reactive oxygen species
RRS	Reduced representation sequencing
RSA	Root system architecture

Rubisco	Ribulose-1,5-bisphosphate carboxylase oxygenase
RWC	Relative water content
SA	Salicylic acid
SCoT	Start codon targeted
SDI	Subsurface drip irrigation
SHMT1	Serine hydroxymethyltransferase 1
SIM	Simple interval mapping
siRNA	Small interfering RNA
SLAF-seq	Specific locus amplified fragment sequencing
SMA	Single marker analysis
SNAT	Serotonin N acetyltransferase
SNP	Single-nucleotide polymorphism
SOC	Suppression of overexpression of constans
SOD	Superoxide dismutase
SOS	Salt overly sensitive
SR	Serine/arginine-rich
SRAP	Sequence-related amplification polymorphism
SSH	Suppression subtractive hybridization
SSLP	Simple sequence length polymorphism
SSN	Sequence-specific nuclease
SSR	Simple sequence repeat
STMS	Sequence-tagged microsatellite site
STS	Sequence-tagged site
SV	Structural variation
TALEN	Transcription activator like effector nuclease
TF	Transcription factor
TFC	Total flavonoid content
TILLING	Targeting induced local lesions in genomes
TIR	Temperature induction response
TLCV	*Tobacco leaf curl virus*
TLP	Thaumatin-like protein
TPC	Total polyphenol content
TRIA	Triacontanol
TRPV	Transient receptor potential cation channel
TSP	Trisodium phosphate
TuMV	Turnip mosaic potyvirus
UHD	Ultra-high-density
UPLC	Ultra-performance liquid chromatography tandem
UPOV	Union for the Protection of New Varieties of Plants
VIGS	Virus-induced gene silencing
WGS	Whole genome sequencing

WT	Wild type
WUE	Water-use efficiency
XET	Xyloglucan endotransglycosylase
ZEP	Zeaxanthin epoxidase
ZFN	Zinc finger nuclease

Chapter 1
Physiological, Molecular and Genetic Analysis of Abiotic Stress Tolerance in Tomato

R. H. Laxman, K. V. Ravishankar, H. C. Prasanna, K. V. Ramesh, K. Rashmi, S. Kannan, K. Hara Gopal, and S. S. Darshan

Abstract Tomato (Solanum lycopersicum L.) being most widely consumed vegetable is grown under diverse climatic conditions. It is exposed to episodes of abiotic stresses at critical stages and the potential yields are seldom realized. Abiotic stresses namely, high temperature, cold, drought, excess and low light, nutrient deficiency and toxicity and salinity limit production. Climate change is expected to further increase the incidence of various abioitic stresses. Under the circumstances it is rational to assess the adverse effects and devise means to overcome ill effects of diverse abiotic stresses on tomato. Tomato improvement efforts for desirable traits like yield and keeping quality have offered results. Evaluation of various tomato germplasm under different abiotic stresses has provided better insights into the existence of diversity in both cultivated and wild relatives. Though, substantial improvements have been made in understanding the abiotic stress tolerance mechanisms and gene discovery, the knowledge has not been exploited extent possible for development of tolerant commercial cultivars. Employing bioinformatics and molecular techniques there is remarkable opportunity for addressing complex breeding problems. Recent molecular tools have greatly helped the scientific community in assessing tomato germplasm for abiotic stress tolerance. Various physiological and biochemical analysis have been employed to understand the mechanisms operating during different abiotic stress tolerance. Using contrasting genotypes, many mapping and genetic studies have been conducted to identify genomic regions linked to abiotic stress tolerance. From these studies, various quantitative trait loci (QTLs) and genes have been identified. The markers could be valuable in improvement programs and for introgression of genes and QTLs from wild type species to Solanum lycopersicum using marker assisted selection (MAS). Many genetic engineering studies concerning transgenic and Crispr/CAS9 have demonstrated the association of various genes involved in imparting tolerance to abiotic stress in tomato. Sol Genomics, a database

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with genetic and genomic information on the plants belonging to Solanaceae family helps in providing sequence and marker related information which can be employed in MAS and genetic engineering. The opportunity for harnessing this information and devising strategies for tomato improvement for abiotic stress tolerance are discussed.

Keywords Abiotic stresses · Tolerance mechanisms · Quantitative Trait Loci · Transgenics · Breeding strategies · Marker assisted selection · Genomics

1.1 Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely consumed vegetables primarily due to its diverse consumption forms either as raw, cooked or various processed products. Its family Solanaceae also includes commercially important vegetables that are grown under diverse climatic conditions. Due to its demand for consumption in various forms, globally it is cultivated for both domestic needs and exports. Globally it is grown in an area of 5.03 million hectares with a total production of 180.76 million tons and productivity of 35.93 tons/ha (FAO 2019). Tomato crop is grown under diverse geographical regions either in open or under protected conditions. Two major tomato growing countries are China and India. Based on the extent of area and requirements it is harvested either manually or mechanically under intensive cultivation. The tomato fruit has diversity in color, shape and size. It is nutritionally important as it contains variety of phytochemicals. The fruit contains red color imparting pigment lycopene, which is a dominant antioxidant. Lycopene is important for maintaining eye health and reduces the incidence of cancer. The fruit also contains other carotenoids (β-carotene, phytoene, phytofluene) and phenolics (coumaric and chlorogenic acids, quercetin, rutinandnaringenin). Vitamin C (ascorbic acid) in reasonable amounts is also present in tomato fruit. An alkaloid tomatine with fungicidal properties too is present. Hence, due its potential nutritional and health benefits, tomato is produced and consumed across the world.

1.2 Effect of Abiotic Stresses on Tomato Growth, Yield and Quality

Though tomato cultivation is widely adopted across the world in diverse agro-ecological regions, the potential yield is seldom realized due to episodes of many abiotic stresses. Abiotic stresses namely, high temperature, cold, drought, excess and low light, nutrient deficiency and toxicity and salinity limit crop production (Criddle et al. 1997; Cramer et al. 2011). Estimates of the effect of abiotic stress on global agriculture suggest that up to 70% of crop production is affected by ecological constraints (Boyer 1982; Cramer et al. 2011). Abiotic stresses are frequently interconnected, occurring either singly or in combination and cause morphological, physiological, biochemical, and molecular changes in plants, reducing the development and production (Bulgari et al. 2019).Tomato requires accessibility of irrigation water throughout the crop growth cycle. It is sensitive to drought stress due to its succulent nature, and occurrence of deficit water stress during flowering and fruit set is very unfavorable. Deficit water stress caused reduction in yield to the extent of 11.69% and 30.60% under mild and severe water stresses, respectively. Conversely, fruit quality in terms of soluble sugars, total soluble solids, vitamin C contents, and fruit firmness was better under water stress (Hao et al. 2019b). The decline in plant height by 24%, stem diameter by 18% and chlorophyll concentration by 32% was observed in tomato under severe water stress condition. Consequently it resulted in 69% lower yield (Sibomana et al. 2013). The overall growth, flowering, yield and mineral nutrient uptake were significantly affected due to deficit irrigation (Ragab et al. 2019).

As a result of erratic rainfall the crops experience excess moisture stress and prolonged waterlogging situations. Such situations manifest into negative influence on crop growth and development. Waterlogging in tomato fields beyond two days leads to complete wilting and yield loss. Waterlogging induced anaerobic condition for 24 h resulted in 15% wilting and 40% yield loss (Hubbell et al. 1979). Flooding stress largely affected the physiology of tomato plants. Under stress tomato plants had lower photosynthesis rate, stomatal conductance, chlorophyll fluorescence, leaf water potential and chlorophyll content (Bhatt et al. 2015).

Maximum tomato rate of fruit growth and yield could be achieved at 25 °C (Adams and Valdes 2002). The critical nature of mean daily temperature on tomato was emphasized by Peet et al. (1997). The reduction in fruit number, per plant fruit weight and seed number was evident at mean daily temperature of 29 °C as compared to 25 °C. Thermal stress in tomato occurs at 35 °C. The exposure of plants to such high temperatures, results in physio-biochemical injury which adversely affect growth and yield (Rivero et al. 2004). High temperature stress during the growth of male reproductive part, leads to reduced fruit formation as a result of interruption in sugar and proline metabolism (Sato et al. 2006). Termination of flowers due to high temperature effect on bud formation and enlargement results in lower tomato yield (Peet et al. 1997; 1998; Sato et al. 2000). Increase in daily mean temperature adversely affects growth and productivity of tomato (Laxman et al. 2013, 2018).

Tomato is moderately sensitive to salinity. Salinity had its adverse influence on tomato root elongation, lateral root growth, reduced leaf, shoot height, stem diameter, photosynthesis and leaf chlorophyll content. At salinity equal and above 5 dS m^{-1} the reduction in total yield was 7.2% per unit increase in salinity (Zhang et al. 2016). Since soil salinity influences seed germination and initial crop establishment, understating its influence is important for tomato production. Salinity level of 3.0% NaCl in Hoagland's solution affected germination of tomato seed. The germination process took longer and higher root/shoot dry weight ratio and Na^+ content with reduction in K^+ content were evident (Singh et al. 2012). At temperatures below 12 °C, the growth and development of tomato cultivars is adversely affected (Hu et al. 2006). Chilling stress damages tomato plants at prevailing temperatures between 0 and 12 °C depending on the duration of exposure (Adams et al. 2001). Thus, the abiotic stresses have a greater bearing on overall physiology, growth, development and yield.

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

Tomato with desirable nutritional and health benefits is in great demand for domestic consumption and export. Increasing population driven demand poses a bigger challenge for sustainable tomato production. Further, there is a pressing need to manage the existing and future extreme weather events anticipated under climate change. The human induced increase in global surface temperature from 1850–1900 to 2010–2019 is likely in the range of 0.8 °C to 1.3 °C. Over the land surface the globally averaged precipitation has likely increased since 1950, with a quicker rate of increase since the 1980s. Currently observed variation in temperature events are projected to become more intense and last longer. The global temperature rise could be managed at 1.5 °C with determined efforts by cutting the emissions. These concerted efforts may prevent the most terrible climate impacts. However, as per the projections, under high emission scenario, the world may be warmer by 4.4 °C by 2100. Under such extreme scenarios the impacts would be devastating (IPCC 2021). Hence, there is an intense challenge of sustaining tomato production in the face of growing world's population on one hand and climate change on the other.

1.4 Limitations of Traditional Breeding and Rational of Genome Designing

Throughout the phenological development, tomato is subjected to a variety of abiotic stresses that adversely affect growth and yield. Among several approaches for sustaining and enhancing yields under adverse stress situations, identificaction of tolerant cultivars is of prime importance. Tomato breeding efforts for various desirable traits, especially yield and keeping quality, have been pursued in the past century employing pedigree method, hybridization, mass selection, and backcrossing. Such concerted efforts have led to improvements in tomato (Lucatti et al. 2013; Iqbal et al. 2019). Hybridization and pedigree selections have been the most important breeding techniques used in tomato improvement. The backcross method of breeding has been used to transfer desirable traits from wild species to cultivated varieties, among several other approaches (Sharma et al. 2019). Over the last seventy years, wild tomato species have been used in breeding programs to improve the cultivated tomato.

Development of tomato cultivars with enhanced abiotic stress tolerance is one of the most sustainable approaches to manage abiotic stresses. In this regard, considerable progress has been made to understand the stress tolerance mechanisms and gene discovery. Despite this there is no report of commercial cultivar tolerant to salt and/or drought stress. The literature on genetic variability, selection indices, important characteristics and genotypic responses to heat stress has been comprehensively reviewed by Hazra et al. (2007). The plant breeding programs need to translate the basic understanding gained from such studies into stress tolerant crop varieties using conventional and molecular tools. Tomato is a model plant for research in the Solanaceae family. The genomic sequence of tomato (*Solanum lycopersicum* L.) and its close wild relative (*Solanum pimpinellifolium* L.) are available (The Tomato Genome Consortium 2012; Aflitos et al. 2014). These advances encourage plant genomics and breeding studies for crop improvement.

Selection and breeding of tomato cultivars that can offer economic yield under abiotic stress situations signifies the lasting and balancing nature of this approach. An effective screening of the diverse genetic material is crucial for successful breeding strategy. The lack of a universal selection criterion for distinguishing tolerant and sensitive tomato accessions necessitates further research into identification of tolerance traits (Hirayama and Shinozaki 2010). The need for enhancing tolerance to abiotic stresses has necessitated the use of both traditional breeding techniques and marker-assisted selection. The evaluation of tomato germplasm under various abiotic stresses has provided better insights into the existence of diversity in both cultivated and wild relatives. However, utilization of diverse genetic resources in breeding tomatoes requires an efficient evaluation of germplasm using the phenotypic and genomic tools (Ayenan et al. 2019).

Following the breakthroughs in molecular biology and bioinformatics, breeding practices have improved (Caliman et al. 2008). These enhance the effectiveness of traditional plant breeding programs. The use of bioinformatics and molecular techniques can improve the screening of complex breeding problems (Ouyang et al. 2007).The narrow genetic basis in cultivated tomatoes for heat tolerance has triggered interest in exploiting tomato wild relatives, which have been sources for many abiotic and biotic stresses and yield related traits (Zhang et al. 2017a). Based on their ability to maintain photosynthetic rate and chlorophyll fluorescence under high temperature, Zhou et al. (2018) identified specific *S. pennellii* and *S. peruvianum* accessions as heat tolerant.

Current tomato breeding achievements are based on traditional breeding-genetic procedures, and limited improvements in introduction of useful traits into cultivars have been achieved. It's reasonable to believe that conventional breeding would not permit production to increase in the future (Fentik 2017). There has been significant development in molecular genetics and the application of molecular marker techniques. As a result, combining conventional breeding with modern plant biotechnology techniques such as marker-asisted selection and selection based on molecular markers could be useful tools for tomato breeding.

1.5 Response to Different Abiotic Stresses

Occurrence of abiotic stresses at critical growth stages not only severely limits productivity and yield but also quality of the produce. Under global climate change situations, abiotic stresses are of vital importance due to their widespread incidence. The occurrence of extreme high temperature and rainfall events are being reported across the world. Various abiotic stresses induce plant responses at different levels namely, morphological, physiological and biochemical/molecular alterations (Raza et al. 2019). At the morphological level, abiotic stress can cause altered shoot, root and leaf growth, as well as developmental changes that result in altered life cycle duration and fewer or smaller organs. Physiological activities such as photosynthetic rate, transpiration, respiration, assimilate partitioning to different organs within the plant, and mineral uptake is affected. At cellular level, membrane disruption, disorganized thylakoid structure, reduced cell size, stomatal guard cell function, alterations in cellular hydration and programmed cell death are manifested (Rao et al. 2016). At biochemical/molecular level, the effects include enzyme inactivation, production of reactive oxygen species (ROS), osmotic damage, changes in primary and secondary metabolite profiles, changed water and ion uptake or translocation and altered hormone concentrations (Etesami et al. 2021).

Maintenance of cell turgidity under stress is crucial for survival and to carry out metabolic activities. Plants have evolved various adaptive mechanisms to maintain positive turgor which mainly involves improving water relations and cellular level tolerance (Kapoor et al. 2020). Desired plant water status is sustained through mechanisms like alterations in phenology, maintaining positive turgor, and eventually sustaining cell metabolic activities despite decreased cell water potential. Cellular responses to stress include adjustments of the membrane system, modifications of the cell wall architecture, and alterations in cell cycle and cell division (Klutz 2005). Therefore, two important relevant mechanisms are (a) improving water relations and (b) improving cellular level tolerance.

1.5.1 Roots Characters

Plants resort to several adaptive strategies through modifications in root characteristics. Such adaptations help to explore and access soil available water and maintain higher water use efficiency. Plants maintain positive turgor by taking up water from the deeper layers of soil (Robbins and Dinneny 2015). The roots act as crucial organ for meeting transpiration demand at a reasonably high leaf water status, given the condition that water is available in the rooting zone. Several root characters have relevance in stress adaptation. Studies have shown that genotypes with desirable root characteristics maintained cooler leaf temperature for longer period under water stress (White and Kierkegaard 2010). Nevertheless, these avoidance features help to maintain higher tissue water content under deficit moisture stress conditions. Such adaptations facilitate plants to delay the immediate adverse effects of water stress. Hence, under severe deficit water stress conditions, the inherent tolerance characteristics are more important (Basu et al. 2016). Plants have exhibited many dehydration tolerance mechanisms under low tissue water potential by maintaining chloroplast integrity, membrane integrity and osmotic adjustment (Parkash and Singh 2020).

The importance of roots in accessing water from deeper layers of soil has also been the primary emphasis by the researchers. Roots, being below ground, respond to incidence of abiotic and biotic stresses in the soil and communicate with the aboveground plant parts via signaling pathways (Kim et al. 2020). The growth and development of plants is controlled by the root morphology and physiology through modifications in root to shoot transport of signaling molecules including hormones, proteins and RNAs and mineral nutrients (DoVale and Neto 2015). Roots being hidden in the soil are frequently exposed to multiple abiotic stresses occurring in the soil. Alterations in the shoot: root ratio is often detected when plants are subjected to various stresses (Fox and Fort 2019). Under drought, salt, or sub-optimal temperature stress, as well as some nutritional inadequacies or elevated levels of atmospheric CO_2, redistribution of metabolites from shoots to roots is frequently observed. Insufficient solar radiation or extra nutrients, on the other hand, usually result in a higher shoot-to-root ratio (Franco et al. 2011).

Roots have a greater ability to sense the physicochemical properties of the soil and change their development and performance, thus playing an important part in the plant's nutritional and development activities under abiotic stress (Kul et al. 2020). Roots are serving as the interface between the plant and the soil, hence more exposed to many abiotic stresses, especially drought, waterlogging and salt stresses than the aerial parts of the plant (He et al. 2018). Drought may cause a greater inhibition of shoot growth compared to root growth and in some cases; the absolute root biomass in drying soil may increase when compared to well-watered soils (Boudiar et al. 2020). Hence, the plants have developed various mechanisms to endure water or salt stress, including an altered shoot: root ratio.

Several studies have provided detailed insights into huge variations among tomato root traits (Table 1.1). The root characteristics like maximum root depth, total root length, root surface area, root volume, root diameter, root length density, root distribution pattern in the soil column, root to shoot ratio, root branching, root hydraulic conductance, root anatomy, root elongation rate, and hardpan penetrability. Intrinsic tolerance of roots plays a major role under stress condition (Sukeshini 2020). Hence, analysis of root phenotypes has revealed better understanding of root growth responses to a variety of environmental stimuli, as well as the extent of natural variation for root traits (Ristova and Busch 2014). Therefore, improving our understanding of the interactions between root functions and abiotic stress tolerance could have a significant impact on adaptation strategies.

Table 1.1 Root traits associated with different abiotic stresses in tomato species

Abiotic stresses	Root traits	References
Drought stress	Root length	Ron et al. (2013), Ghebremariam et al. (2013), Khan et al. (2014), Senthilkumar et al. (2017), Habib et al. (2019)
	Root biomass	Brdar-Jokanovic et al. (2014), Tron et al. (2015), Mahpara et al. (2018), Buhroy et al. (2017), Zhang et al. (2019), Zhang et al. (2020)
	Root/shoot ratio	Allerstorfer (2014), Niakan et al. (2014), Xiong et al. (2015), Zhang et al. (2020), Kamanga et al. (2020)
Salt stress	Root length	Almutairi, (2016), Tanveer et al. (2020), Habib et al. (2019), Altaf et al. (2020)
	Root biomass	Singh et al. (2012), Sajyan et al. (2018)
	Root/shoot ratio	Ebrahim et al. (2017), Parvin et al. (2019), Ladewig et al. (2021)
High temperature stress	Rootlength, Root biomass Root/shoot ratio	Keatinge et al. (2014), Haghighi et al. (2014), Shaheen et al. (2016), Ali et al. (2020)
Cold stress	Root length	Zhang et al. (2011), Subramanian et al. (2016), Wani et al. (2021)
	Root biomass	Ghorbanpour et al. (2018), Dezhabad et al. (2020)
	Root/shoot ratio	Klay et al. (2014), Altaf et al. (2021)

1.5.2 Heat Tolerance

As a result of global warming, coincidence of high temperature episodes with sensitive phenophases, leads to heat stress and is a major agricultural concern in many parts of the world. Heat stress is a vital limiting factor in agricultural output. There is a spike in air temperature over a threshold level for a period of time long enough to produce injury or irreversible damage to crop plants in general (Kumar et al. 2012; Lobell and Gourdji 2012; Gourdji et al. 2013; Teixeira et al. 2013). The extent of high temperature induced alterations in physiology, phenology, growth and yield depends on the duration, intensity and temperature increase over the threshold limits. The effects of heat stress on plants are very complex; resulting in denaturation of enzymes, disruption of metabolism activity, growth and development alterations, change in physiological functions and morphological structure (Mondal et al. 2013). Such alterations results in increased respiration, lower photosynthesis rate, closure of stomata, reduced chlorophyll fluorescence, lower chlorophyll content, membrane damage, ROS over production, and metabolic disruption upon exposure to heat stress (Hu and Zhu 2020). It could also alter the phenology, shorten the crop duration, days to flowering and fruiting, hasten fruit maturity, ripening and senescence. These alterations result in reduced crop productivity and quality (Yu et al. 2019).

Tomato is cultivated in diverse agro-climatic regions across the world. It is very sensitive to high temperature (Camejo et al. 2005). Optimum mean daily temperature requirement is between 15–32 and 35 °C is considered as maximum threshold (Zhang et al. 2005). For growth, development and yield 40 °C is supra-optimum temperature (Morales et al. 2003). High temperature can undesirably impact seed germination, vegetative growth, pollination, flowering, fruit set, fruit weight and fruit quality (Foolad et al. 2005; Laxman et al. 2018). The gas exchange characteristics, photosynthesis, transpiration, stomatal conductance and photochemical efficiency of PSII were affected to a greater extent at peak flowering stage as compared to peak fruiting stage (Camejo et al. 2005, 2006; Laxman et al. 2013, 2014).

Previous studies have shown that the increase in daily mean temperatures adversely affect growth and productivity of tomato. The increase in temperature above the optimal, caused reduction in net photosynthetic rate, transpiration, stomatal conductance, and photochemical efficiency of PSII of five selected tomato genotypes during peak flowering and fruiting stages. The reductions were higher at peak flowering stage compared to peak fruiting stage (Laxman et al. 2013). The mild temperature also reduced in vitro pollen germination, fruit set percentage, fruit weight, number of leaves, number of branches, plant height, total dry matter accumulation, and harvest index in tomato plants. Concurrently, the number of trusses, flowers, and flower abortion also increased (Laxman et al. 2018). Antioxidant enzymes and the expression of HSPs/HSFs genes were found to be involved in the SlMAPK3-mediated heat stress response in tomato plants Yu et al. (2019). Mansy et al. (2021) studied six tomato lines, G1, G2, G3, G4, G5, and G6, under heat stress at the morphological, molecular, and cytological levels. The lines G2, G1, and G6 performed better in terms of morphological characters, vegetative development, fruiting, and yield.

In order to sustain productivity and quality under climate change situations, identification of tolerance source and development of suitable cultivars is the best adaptation strategy. For sustainable crop production under heat stress, two most imperative strategies could be followed: (a) introduction of tolerant cultivars, genetically modified or transgenic cultivars through molecular and biotechnological means along with conventional breeding approaches and (b) employing several agronomic management strategies for heat stress management under field conditions. As part of the strategy, coping up with high temperature stress under climate change conditions necessitates development of tolerant cultivars. This can be accomplished by breeding programs by exploiting the genetic capability of genotypes which are already temperature tolerant (Laxman et al. 2018). Bhattarai et al. (2016) examined a tomato cross to estimate combining ability and understand the genetic basis of tomato genotypes under heat stress. The genetic components and proportions studies showed that the heat tolerance was governed by non-additive gene action and is a dominant trait. The parental variance (*Vr*) and parent–offspring covariance (*Wr*) relationship (*Wr*–*Vr*) indicated that heat tolerance traits were governed by over dominance. Significant correlation occurred between yield and yield-attributing traits. Heterosis was high for yield and most yield-attributing traits. Selection criteria should be to select heat tolerance traits in early generations followed by selection for yield.

The accurate and thorough understanding of reproductive key traits (flowers with exerted stigma, pollen viability, fruits set per cent and number of fruits per cluster), along with earliness (days to first fruit set) and yield traits (average fruit weight, pericarp thickness, number of fruits per plant and yield per plant) for heat tolerance of tomato is very essential. Insights of genetic architecture of the reproductive traits under heat stress might improve core understanding and might have applied significance. Therefore, new breeding lines and breeding strategies can be implemented under changing environmental conditions with special reference to elevated temperature (Archana et al. 2021).

Attempts have been made to evaluate the diverse tomato genotypes and assess the traits responsible for high temperature stress tolerance. Employing temperature induction response (TIR) technique, among the 52 tomato genotypes, 21 were identified as tolerant, 12 moderately tolerant and 19 genotypes as susceptible (Geeta et al. 2019). Such screening techniques that are simple and quick could be easily adopted in crop breeding programmes. In a study involving forty-nine diverse tomato genotypes, the tolerant genotypes exhibited lower reduction in photosynthesis rate, chlorophyll fluorescence transients, relative water content, membrane stability index and yield traits (number of fruits and fruit weight) with higher accumulation of osmolytes (Geeta 2020). Hence, breeding for high temperature tolerant crops needs high priority (Driedonks et al. 2016). In this endeavour, it is essential to fully characterize and identify genetic variation with respect to high temperature tolerance traits in the available germplasm before using them in a breeding program.

1.5.3 Cold Tolerance

Low temperature stress is categorized into chilling (<20 °C) and freezing (<0 °C) stresses. In addition to affecting the growth and development of the plants, cold stress significantly restrains the geographical distribution of plants (Liu and Zhou 2018). Tomato is an important thermophilic crop that is being cultivated in many areas worldwide (Yang et al. 2017). Adverse effects of cold stress on tomato caused reductions in yield and quality (Zhang et al. 2021). Cold stress adversely affects plant growth and development throughout the ontogeny of the tomato plant. During seed germination it delays the onset, reduces the rate, and increases the spread of germination events, resulting in poor stand establishment and crop performance. At later stages, cold stress results in reduced plant growth and development, delayed flowering, production of sterile pollen, low fruit set, and substantial reduction in fruit yield (Foolad and Lin 2000).

Plants must retain cell functionality and activity under cold stress, especially the stability of the cell membrane and the structure of the protein with biological activity, in order to survive in adverse environments. When plants are exposed to subzero temperatures, ice forms in the tissues. Plants with higher levels of active ice nucleators in their apoplastic solution have a higher freezing point (Ritonga and Chen 2020). Developing cold-tolerant cultivars is one way to reduce the harmful consequences of cold stress. A cold-tolerant tomato cultivar should have the ability to germinate rapidly and uniformly, grow efficiently, produce flowers, and set fruit at low temperatures (Foolad and Lin 2001). At 4 °C low temperature, two wild *S. habrochaites* species, the IL LA3969 and its donor parent LA1777 were more cold tolerant than the IL's of recurrent parent *S. lycopersicum*, LA4024 (Liu et al. 2012).

Tomato plants treated with psychrotolerant bacteria isolates showed significant tolerance to chilling stress, as seen by reduced membrane damage and antioxidant enzyme activity, as well as proline synthesis in the leaves when subjected to chilling stress conditions. Their ability to promote germination, growth, and induce antioxidant capacity could help protect plants from chilling stress (Subramanian et al. 2016). As proposed by Liu et al. (2020), soluble carbohydrates influence tomato plant growth and development indirectly by serving as signal molecules in processes that increase tolerance to cold stress. Raffinose family oligosaccharides (RFOs) are an example and play an important role in cold stress tolerance. As a signal molecule in the activation of cold tolerance, galactinol plays an important role in RFO production as a critical limiting factor. Expression of galactinol synthase, *AnGolS1* in tomato enhanced cold tolerance and led to changes in the sugar composition of the seeds and seedlings.

As measured by free proline, membrane stability index, and PS II activity, transgenic tomato lines had a higher level of resistance to cold than normal plants (Parmar et al. 2017). Abscisic Acid (ABA) plays an important role in the induction of late embryogenesis abundant (LEA) gene expression (Lin et al. 2021). In plant vegetative tissues, exogenous ABA promoted the expression of certain LEA genes. ABA increased the expression of LEA genes during seed development and during abiotic stress exposure (Atayee and Noori 2020). The role of ABA in the up-regulation of LEA genes is considered to be one of the mechanisms that ABA increases freezing tolerance in tomato plants (Sah et al. 2016). Gibberellic acid (GA) is an important component of the plant's growth-control mechanisms and is altered in cold-stressed plants. GA has been observed to play a role in the expression of the CRT/DRE-binding factor gene, which provides cold stress resistance in plants (Atayee and Noori 2020). The lower expression of key GA metabolic genes, GA3ox1, GA20ox1 and GA2ox1 and GA3 treatment reduced the chilling injury index in tomato plants (Zhu et al. 2016).

1.5.4 Flooding Tolerance

The present weather patterns show occurrence of extreme rainfall events. Such extreme events are predicted to be more frequent under climate change conditions. The change in rainfall patterns would lead to excess and deficit moisture stresses at various stages of the crop growth. Excess moisture stress can alter the physio-chemical properties of the soil which have an adverse effect on the plant growth and development. The physical and chemical characteristics of soil, such as redox potential, pH, and oxygen content, are all affected by water logging (Pandey et al. 2021). Shortly after the soil is flooded, the respiration of roots and micro-organisms depletes the remnant oxygen and the soil environment becomes hypoxic (i.e. oxygen levels limit mitochondrial respiration) and later anoxic (i.e. respiration is completely inhibited (Bailey-Serres and Voesenek 2008; Wegner 2010). Hence, the first constraint for plant growth under flooding is the immediate lack of oxygen necessary to sustain aerobic respiration of submerged tissues (Vartapetian and Jackson 1997; Striker 2012; Mignolli et al. 2021).

The earliest detectable physiological symptoms of flooding stress include decreased photosynthesis rate, stomatal conductance, transpiration rate, net assimilation rate and root hydraulic conductivity (Doupis et al. 2017). Ethylene directs the response of tomato to flooding stress; hypoxia that accompanies flooding causes an increase in the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase in roots, as the expression of ACC synthase genes is induced and ACC oxidation is arrested in roots (Klay et al. 2018). Despite the fact that tomato plants can thrive in a variety of climes, they are one of the most vulnerable vegetable species to excessive soil wetness. Heavy rain combined with poor drainage causes water logging, which limits oxygen levels in the soil, resulting in plant mortality. Water stagnation in tomato fields for 2–3 days results in considerable crop loss and in some cases, complete plant death (Bhatt et al. 2015). Several studies have reported the antagonistic effect of excess moisture stress on plant growth and productivity. The findings of Ezin et al. (2010) revealed that the chlorophyll fluorescence and relative chlorophyll content act as a good criterion in the selection for flooding tolerance in tomato. Flood tolerant species are able to produce a lot of adventitious roots and sustain fruit yields. CLN2498E and CA4 genotypes were highly, and LA1421 moderately tolerant to flooding, and LA1579 was not tolerant to flooding. These flood tolerant genotypes could be used as good candidate lines in molecular breeding program to establish definite relation with chlorophyll fluorescence, chlorophyll content, yield and yield components. The effect of attenuated ethylene sensitivity in the Never ripe (Nr) mutant on leaf photosynthetic capacity of flooded tomato plants showed reduced ethylene perception in Nr plants. This was associated with a more efficient photochemical and non-photochemical radiative energy dissipation capability in response to flooding. The response was correlated with retention of chlorophyll and carotenoids in flooded Nr leaves. Therefore, besides its role as a trigger of many adaptive responses, perception of ethylene entails limitations in light and dark potential efficiency of PSII photochemistry; stomatal conductance; non-photochemical quenching; photosynthetic photon flux density, maximum rate of Rubisco-mediated carboxylation; intrinsic water use efficiency; actual photon yield of PSII photochemistry (De pedro et al. 2020).

When partially submerged, tomato plants undergo profound changes involving rearrangements in their morphology and metabolism; it markedly affects root respiration and halts root growth. Root sink disruption presumably causes sugars to accumulate in hypocotyls. As the availability of substrate (sucrose) increases, respiration is fueled creating a sucrose gradient that results in a sustained transport of carbohydrates to the submerged hypocotyls (Mignolli et al. 2021). In this sense, remobilization of accumulated sugars in the hypocotyl could sustain root system growth resumption when flooding water recedes. Girdling and feeding experiments point to sucrose as the main carbon source for respiration. Consistently, submerged hypocotyls are characterized by high sucrose synthase activity, indicating that sucrose is cleaved and channeled into respiration (Mignolli et al. 2021).

Excess moisture condition can be overcome by identifying tolerant genotypes for sustainable tomato production (Ezin et al. 2010; Lin et al. 2016; Tareq et al. 2020; Mignolli et al. 2021). Inter-specific grafting approach to excess moisture condition has been reported in several studies (King et al. 2010; Keatinge et al. 2014; Bhatt et al. 2015; Bahadur Anant et al. 2015; Lin et al. 2016). Tomato and eggplant (*Solanum melongena* L.) plants differ in their resistance to flooding condition and have different physiological mechanisms to overcome flooding stress. Eggplant roots are relatively tolerant to flooding stress, survive better under excess water and exhibit better graft compatibility with tomato (Aganon et al. 2002; Black et al. 2003; Bhatt et al. 2015).

To overcome the deleterious consequence of flooding stress many studies emphasized on genetic modification approach through identification of gene and development of transgenic plants in tomato (Grichko and Glick 2001; Huther et al. 2016; De Pedro et al. 2020; De Ollas et al. 2021). As roots are the first organ to be affected by hypoxia, the ability to sense and respond to hypoxic stress is crucial. At the molecular level, therefore, fine-tuning the regulation of gene expression in the root is essential for hypoxia tolerance. Using an RNA-Seq approach, transcriptome modulation in tomato roots were examined and results suggested that the regulated genes identified are good candidate genes for hypoxia tolerance in tomato (Ivanchenko et al. 2020; Safavi-Rizi et al. 2020).

1.5.5 Water Use Efficiency

Water is essential for crop production across the world. Climate change triggered unpredictable rains cause frequent droughts. Such unusual rainfall patterns enforce crop cultivation under assured irrigation. The overdependence on ground water and other fresh water resources for crop production has resulted in depletion of freshwater available for irrigation. These circumstances, demand water-efficient crop growing practices. Enhancing agricultural water-use efficiency and sensible water use in crop production may help in realizing “more crop per drop” (Farooq et al. 2019). Water use efficiency (WUE) describes the intrinsic trade-off between carbon fixation and water loss that occurs in dry land plants because water evaporates from the interstitial tissues of leaves whenever stomata open for CO_2 acquisition. The transpiration efficiency of crop plants is generally low as they typically lose several 100-fold more water than the equivalent units of carbon fixed by photosynthesis (Bramley et al. 2013). With the increasing demand for sustainable water use and increasing agricultural productivity, the need to improve transpiration efficiency of crops has received much attention, although this trait may not be beneficial in all water-limited environments.

WUE may be estimated as the ratio between net photosynthesis (Pn) and transpiration (E), which is known as instantaneous water use efficiency (physiological index) (Polley 2002). The ratio between Pn and stomatal conductance (gs), is known as intrinsic water use efficiency (Lei 2018). And the ratio of dry matter accumulation over time to the amount of water transpired is known as biomass/yield water use efficiency. A large diversity in WUE has been reported among different crops and genotypes. The limited water availability situations for crop production could be managed through increase in WUE. Wild tomato species are genetically diverse and exhibit a range of tolerances to one or more abiotic stresses. Wild species, including *S. habrochaites*, have been reported to exhibit increased WUE when compared to cultivated tomato (Zsogon et al. 2017). Deficit irrigation resulted in higher water use efficiency compared to full irrigation in case of fresh and dry tomato fruits (Djurovic et al. 2016). Changes in WUE under full and deficit irrigation conditions were also reported by Topcu et al. (2007), Patane and Saita (2015), Wang et al. (2015). The limited water availability situations for crop production could be managed through increase in WUE. Wild tomato species are genetically diverse and exhibit a range of tolerances to one or more abiotic stresses. Tomato yield and WUE increased asymptotically with irrigation amount before approaching their plateau when the irrigation amount reached 90% and 70% of accumulative evaporation, respectively. While the yield was positively related to fruit size and negatively to soluble solid content, this was independent of WUE (Liu et al. 2019). WUE significantly increased under non-loamy soil while not in loamy soil. Under regulated deficit irrigation (RDI) WUE increased significantly and was beneficial if RDI was applied to tomato in non-loamy soil, since reductions in yield were lower and water use efficiency higher (Lu et al. 2019). Subsurface drip irrigation (SDI) with air injection act as a useful strategy to the reduce evapotranspiration (ET) and the improvement of WUE of tomato (Wang et al. 2020a).

To address nutrient deficiencies by root due to water deficit condition, foliar fertilization with water soluble Tecamin flower®, a product containing chemicals were utilized to evaluate impacts of foliar application at 0 or 2.5 ml/l and deficit irrigation (50 or 100% of field capacity) in tomato (*Solanum lycopersicum* L.) cvs. Bobcat, Finenss and Hadeer. The plant treated with 2.5 ml/l Tecamin flower® produced the highest vitamin C content, total soluble solids, titratable acidity, fruit firmness, total yield, WUE and the lowest pH. Foliar application of Tecamin flower®, regardless of cultivar, improved production, fruit quality and WUE of tomato under normal and water deficit conditions and played a role in alleviating the negative impact of water deficit (Al-Shammari et al. 2020). Hence, studies on response of tomato genotypes and assessment of WUE not only helps in understanding the diverse physiological response of tomato genotypes to water stress but consequently in realizing sustained yields under water limiting conditions.

1.5.6 Nutrient Use Efficiency

Nutrient use efficiency (NUE) is a critically important concept for evaluating crop production systems and can be greatly determined by fertilizer management as well as soil- and plant-water relationships. NUE is a measure of how best plants utilize the available mineral nutrients in the soil as well as from applied source of nutrients in the form of fertilizers. It can be defined as yield (biomass) per unit input (fertilizer, nutrient content) (Fixen et al. 2014). It is one of the complex traits: it depends on the ability of plant to take up nutrients from the soil, but also on transport, storage, mobilization, usage within the plant, and even on the environment. Improved NUE is not just a prerequisite for advancing crop production into relatively low soils, but it is also a strategy to reduce inorganic fertilizer consumption (Reich et al. 2014). NUE is of particular interest as a major target for crop improvement.

The tomato genotype, LA-2157 having ability to use nitrogen efficiently in terms of production of biomass per unit of applied nitrogen under both high nitrogen source and low nitrogen source media was identified. It confirms the availability of source for exploitation in future tomato breeding programmes (Jasmitha 2020). Evaluation for absorption and utilization of phosphorus in three tomato genotypes at different levels of P_2O_5 showed that the genotype, Globonnie had greater efficiency of phosphorus absorption and utilization applied at lower doses (Marques et al. 2018). An anthocyanin-free tomato genotype H957could tolerate lower phosphorus concentration by utilizing internal P with better efficiency rather than by better absorption of external phosphorus (Lee et al. 1998). The genotypes, *Solanum pimpinellifolium, Solanum peruvianum, Solanum galapagense, Solanum arcanum,* PKM-1, Arka Samrat were identified having higher phosphorus acquisition efficiency (Soumya 2020).

The development of new strategies to overcome production failure and the improvement of NUE are dependent on management practices. Quantifying the true status of nutrient use efficiency in agriculture however remains, difficult as reliable farm level data are not widely available (Dobberman 2007). Abiotic stresses and nutrient deficiencies in the soil are two important environmental factors that affect plant growth, productivity, and quality. Earlier studies suggested that grafting approach act as a tool to improve NUE in tomato plants. Where, selection criteria for the breeding of rootstocks help to enhance NUE of elite tomato cultivars under a wide range of growth (root) environment conditions (Venema et al. 2011). Grafting studies indicated that rootstocks affect leaf macronutrient content and enhance nutrient uptake and NUE (Rivero et al. 2005; Leonardi and Giuffrida 2006).

1.5.7 Drought Tolerance

Moisture deficit stress has a major adverse effect on plant growth and development in terms of alterations in physiology, growth, metabolism and production. Extent of damage varies with the level of plant tolerance (genotype), duration, timing and intensity of the stress. Moisture stress affects plant water relations, reduced water content, stomatal movement, limits gaseous exchange, reduces transpiration, reduces carbon assimilation (photosynthesis) rates, mineral nutrition (uptake and transport of nutrients) and metabolism. It leads to a reduction in the leaf area and thus altered assimilate partitioning among the different parts of plants.

Tomato is sensitive to drought stress at all stages of plant growth and development. In terms of productivity, the transplanting, flowering and fruit setting phases have been considered to be the most susceptible phases. Tomato plants growing under moisture stress are generally associated with slow growth, and in severe cases, dieback of stems and death. Such plants are more susceptible to disease and less tolerant of insect feeding. The germination and initial seedling establishment are the first to be adversely affected under scarcity of water (Hamayun et al. 2010). Deficit moisture at germination stage will delay and reduce germination and under severe conditions, may even completely hinder the seed germination. Reduction in seed germination to an extent of 40–60% can be observed under deficit moisture conditions (Ayaz et al. 2015).

Drought stress in tomato is also associated with increased salinity levels which reduce seed germination. It affects both cell elongation and expansion during plant growth and development resulting in reduction in plant height. Under severe water deficiency, cell elongation can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami 1998). Water deficit stress caused impaired mitosis, cell elongation and expansion resulting in reduced growth and yield traits (Hussain et al. 2008). Plant height could reduce up to 58% under severe stress conditions (at 35% moisture of field capacity). Low moisture stress condition reduces tomato growth cycle by accelerating different growth and development stages. With the onset of drought stress, plant developmental phase is stimulated to shift from vegetative to reproductive phase. Relative water content (RWC) and electrolyte leakage are the indicative of metabolic activities within plants and are used for evaluation of plant tolerance to different abiotic stresses including drought. Under stress conditions, RWC decreases and electrolyte leakage increases with increasing stress levels (Ullah et al. 2016).

Tomato plants require a consistent supply of water throughout the growing season in order to achieve maximum quality and output. Tomato plants should be irrigated frequently with small amounts of water and need to receive water between 400 and 600 mm during the growth period. The plants should not be allowed to consume more than 40% of the available moisture in the soil. Water stress at an early stage of development (20 days) is more inhibitory than at a later stage (30 days) (Shamsul et al. 2008). Further, photosynthesis is hampered by stomatal closure and metabolic damage caused by a lack of water. Plant hormones play a critical role in the internal signaling network, and regulate plant growth and development in response to external signal cues (Liu and He 2017). Under water stress conditions, concentrations of ABA, hormone responsible for stomatal closer under water stress condition, can increase up to 50 times. Higher levels of Zeaxanthin epoxidase (ZEP) transcript levels, an important component of ABA synthesis which catalyzes the synthesis of violaxanthin, were observed in root but not in leaves of tomato plants during drought stress. They initiate signal transduction under stress conditions and further regulate stomatal behavior and reduce transpiration rate by closing stomata (Thompson et al. 2000). ABA also regulates root-shoot ratio of tomato plant and stimulates higher root growth as compared to shoot under water stress condition (Sharp et al. 1988).

Water stress stimulates excessive production of ROS. The increased activity of antioxidant enzyme defense system, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), is an adaptive response of plant to drought tolerance. In tomato plants, the activity of SOD was more correlated to drought stress than those of ascorbate peroxidase or catalase (Aghaie et al. 2018). Proline accumulation is a significant response of plants under drought stress conditions. PEG induced drought stress has shown to enhance endogenous proline concentrations in tomato calli (Shtereva et al. 2008).This osmolyte acts as a scavenger of OH^- radical and plays an important role in osmotic adjustment during oxidative stress (Anjum et al. 2000). Further, Proline assists in sustained root growth under water stress condition. It accumulates in root growing zone and increases the activity of enzyme such as xyloglucan endotransglycosylase (XET) and accelerates cell elongation by cell wall loosening (Hartung et al. 1999) inducing root growth under drought stress.

Drought stress severely hampers the gas exchange characteristics of crop plants and this could be due to decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence, oxidation of chloroplast lipids and changes in structure of pigments and proteins (Menconi et al. 1995). Under mild drought stress, inhibition of photosynthesis is due to stomatal closure caused by the imbalance between light reaction and Calvin-Benson cycle as a consequence of limited CO_2 diffusion into the leaf (Chave et al. 2009). Under severe water stress conditions damage to photosystem, inhibition of Rubisco and other enzyme activities lead to decrease in photosynthesis rate (Yuan et al. 2016).The net photosynthetic rate declines distinctly with the decrease in transpiration rate and stomatal conductance with the prolonged water stress. Further, the stomatal length and stomatal width decreases with increase in stomatal density during the stress conditions (Hao et al. 2019a). With the increase in the degree of drought stress, chlorophyll fluorescence characteristics gradually decrease, due to the closure of the PSII reaction center, limiting electron transfer and reducing the light energy available for actual photochemical reactions in the PSII reaction center leading to reduced photosynthetic rate (Table 1.2).

During drought stress conditions, several genes are activated which lead to physiological and metabolic changes against the stress perceived. Drought tolerance is a polygenic trait involving several genes via a complex mechanism. Drought induced genes are regulated by a specific signal transduction pathway which activates transcription factors. These activated genes are involved in protection with creation of efficient antioxidant system, water channels/ transporters, key enzymes for osmolyte biosynthesis (proline, sugars), protection factors for new molecules (LEA proteins, chaperons), ABA biosynthesis, etc. (Shinozaki and Shinozaki 2007) (Table 1.3).

Table 1.2 Impact of drought on photosynthetic parameters in tomato plants

Photosynthetic parameter	Impact	References
Net CO_2 assimilation rate	Decrease	Rao et al. (2000)
Transpiration rate	Decrease	Hao et al. (2019a)
Stomatal conductance	Decrease	
Intercellular CO_2	Increase	Rao et al. (2000), Hao et al. (2019a)
Transpiration	Decrease	Hao et al. (2019a)
Stomatal limitation	Increase	Liang et al. (2020)
F_V/F_M	Decrease	Liang et al. (2020)
NPQ	Initially increase later decrease	Liang et al. (2020)
Rubisco (rbcs)	Decrease	Bartholomew et al. (1991)
Chlorophyll content	Decrease	Ghorbanli et al. (2013)

Table 1.3 Characteristics of genes potentially involved in stress response in tomato

Gene	Characteristics/Description	Function	References
SlGATA17	GATA transcription factor	Regulate the activity of the phenylpropanoid biosynthesis pathway	Zhao et al. (2021)
JUB1	NAC transcription factor	Activation of *DELLA* and the stress-related genes *DREB2* and *DREB1*, reduced ROS levels	Thirumalaikumaret al. (2018)
SL-ZH13	Zinc finger-homeodomain proteins	Increased antioxidant activity	Zhao et al. (2019)
HsfA1a	heat-shock transcription factor	Activate ATG genes and induce autophagy	Wang et al. (2015)
AnnSp2	Annexin; Calcium-dependent phospholipid-binding proteins	ABA synthesis and the elimination of ROS	Ijaz et al. (2017)
BES1	BES1 transcription factor	BR signaling transductionand the elimination of ROS	Wang et al. (2020c)

The limited water availability situations for crop production could be managed through increase in WUE. Wild tomato species are genetically diverse and exhibit a range of tolerances to one or more abiotic stresses. Wild species of *S. pimpinellifolium*, *S. pennellii*, *S. habrochaites*, *S. chmielewskii*, and *S. cheesmanii* are shown to have tolerance to drought stress and they can be suitably exploited for transfer of genes to cultivated species (Solankey et al. 2015). The *S. chilense* is five times more tolerant to wilt than other cultivated tomato. *S. habrochaites* and *S. pennellii* have been reported to exhibit increased WUE when compared to cultivated tomato. These species possess well-developed, longer, primary roots and more extensive secondary roots than cultivated tomato and have thick, round, waxy leaves having acyl-sugars in the trichomes (Easlon et al. 2009; Nicoleta and Nedelea 2012).

1.6 Breeding for Abiotic Stress Management in Tomato

1.6.1 Salt Stress

Salinity in tomato affects different aspects of the physiology and biochemistry of plants and thus significantly reduces yield. There are comparatively more salt tolerant wild relatives of tomato. Because of the large number of genes involved in salt tolerance, it is difficult to enrich elite lines with genes from wild species that confer tolerance. If it is possible to unravel the molecular markers tightly linked to the genes governing salt tolerance, their favourable alleles could be selected in segregating populations using tightly linked markers and eventually incorporated into salt-tolerant cultivars.

Tomato wild relatives are important sources of genes and salt tolerant traits. Wild relatives such as *S. peruvianum*, *S. chilense*, *S. habrochaites*, *S. pimpinellifolium*, *S. cheesmaniae*, and *S. pennellii* are known to be less sensitive to saline growth conditions. The QTL analysis of salt tolerant accessions of *S. pimpinellifolium* and *S. cheesmaniae* led to the discovery of highly significant QTLs (>40%) on chromosome 7 that contribute to salt tolerance in terms of Na^+ and K^+ concentration. The candidate genes of tomato *LeNHX3* gene and two other high affinity potassium transporters (*HKT1*) are located on chromosome 1 and chromosome 7, respectively (Villalta et al. 2008). The tomato *LeNHX3* gene contributed to higher accumulation of leaf Na^+ and the QTLs of *S. cheesmaniae* contributed to build up higher levels of Na^+ and/or lower K^+/Na^+ ratio in leaves. The markers linked to such contributing alleles can be used in marker assisted introgression. The genome-wide markers available now due to advances in genomics can be used for mapping QTLs contributing to salt tolerance using linkage analysis or association mapping (AM) studies. Using AM studies, two important genes of *S. pimpinefolium* namely *AVP1* encoding vacuolar H^+-pyrophosphatase and *LeDREB1A* were identified as critical genes for salinity tolerance (Rao et al. 2015).

The selections were made for salinity tolerance at germination, seedling establishment and reproductive stage from the progenies of cross between *S. cheesmaniae* and tomato cultivar *S. lycopersicum*. The selected progenies were tested for survival and fruit production in salinized solution culture experiments and greenhouse trials. The plants selected from the F2 and successive backcrosses to the cultivar survived and produced fruit when irrigated with up to 70% sea water in the sandy soil culture trials, whereas tomato cultivar did not survive in saline conditions (Shah et al. 2008). The over expression of a known multiple stress responsive gene proline-, lysine-, and glutamic-rich type gene SpPKE1 isolated from *S. pennellii* enhanced the salt tolerance in tomato. Therefore, SpPKE1 and *S. pennellii* could be an important candidate gene and a species for molecular breeding of salt-tolerance in tomato (Li et al. 2019).

1.6.2 Heat Stress

Tomato is sensitive to heat stress. The vegetative and reproductive growth of tomato is impacted by heat stress which can lead to significant total yield loss. Therefore, it is imperative to identify heat tolerant genotypes and develop heat tolerant cultivars. There is significant genetic variability in tomato germplasm with regard to heat tolerance (HT) that can be harnessed to breed heat tolerant cultivars. The essential components of breeding heat tolerance in tomato are screening germplasm, trait breeding for target traits and MAS. Screening germplasm for HT is a critical component. The screening can be carried out under controlled environment conditions such as phytotrons, growth chambers, and greenhouses. However, the applicability of the findings of controlled environment conditions needs to be tested and verified in field conditions. Alternatively, screening at field conditions can also be initiated in parallel to complement the findings of controlled environment studies. The GxE interaction in the field conditions could also play an important role in response to heat stress. Therefore, it is always desirable to screen germplasm in multi-environment conditions including seasons and locations.

The trait breeding strategy may have to be adopted to enhance HT in cultivars. The traits with high heritability should be targeted to achieve better genetic gain in terms of HT. Several studies have focused on morphological, reproductive, physiological and biochemical traits. In general, these studies have focused on male reproductive traits (pollen viability, pollen number and anther dehiscence), female fertility traits (stigma receptivity and seed set after artificial pollination), biochemical traits (soluble sugars, osmolytes, flavonoids and pectins), membrane thermo stability and physiological traits (chlorophyll fluorescence, canopy temperature and transpiration rate) (Ayenan et al. 2019).

Deploying MAS is critical for improving the accuracy and efficiency of HT breeding. This depends on the availability of reliable marker and trait linkage or association. In this regard, advanced backcross populations and chromosome segments substitution lines have been used to exploit genetic variability that exist in non-cultivated tomato species. Several bi parental mapping studies have been conducted so far. The genetic basis of variation in HT of a tolerant line Nagcarlang was investigated previously in a F2 mapping population under continuous mild heat conditions. Then the QTLs were identified for a number of traits related to reproductive success including a highly significant QTL which accounted for 36% of phenotypic variation in the population for pollen viability (Xu et al. 2017). The A meta quantitative trait loci (MQTL) analysis of four mapping experiments led to the conclusions that QTLs associated with heat tolerance traits might show pleiotropic effects on HT or tight linkage of QTLs of HT traits which was evident from the co-localization of QTLs

(Ayenan et al. 2019). Aside from fruit set percentage, fruit weight is also negatively impacted due to heat stress.

Therefore, understanding basis of negative association between fruit weight and thermo-tolerance is required. This aspect of fruit development requires attention since the overall improvement of yield under heat stress will depend on fruit number and fruit size. In an association mapping experiment, six yield-related traits including flower earliness, number of flowers per inflorescence, fruit set, number of fruit per plant, fruit weight and yield per plant were studied (Ruggieri et al. 2019). A total of 15 common markers associated with these traits were identified. The most relevant associations co-localized with genes involved in the floral structure development, such as the style 2.1 gene, or with genes directly involved in the response to abiotic stresses. These promising candidate genes could be used as potential genes to improve tomato cultivar performance under high temperatures.

1.6.3 Drought Stress

There is great deal of genetic variation in tomato for drought stress resistance mostly in wild relatives. Among the tomato wild species, *S. pennellii, S. chilense* and *S. sitiens* are known drought-adapted species. The *S. pennellii* has been used as an experimental model to gain deeper insights in to the underlying molecular mechanisms of drought adaptation and tolerance in tomato (Atarés et al. 2011). The availability of genome sequence, introgression library and backcross inbred lines has assisted in the identification of mechanisms involved in stress tolerance including other traits of importance.

The physiological level changes observed between *S. pennellii* and cultivated tomato demonstrated that *S. pennelli* exhibits reduced stomatal conductance and transpiration rate thus avoid leaf water loss under drought conditions. Also, comparatively *S. pennellii* exhibits lower stomatal density and aperture. The genes involved in amino acid metabolism and ethylene/Jasmonate pathways are elucidated as key factors in the drought tolerance of *S. pennelli* (Egea et al. 2018). The natural variation that exists for plant water use efficiency (WUE) may be explored as it can contribute to yield under water limited conditions. The carbon isotope composition is being used as measure of plant WUE as both vary in concert in C3 plants. A dominant QTL for carbon isotope composition was detected on chromosome 5 using introgression lines of *S. pennellii*. The markers linked to this QTL can be used in MAS for enhancing WUE in tomato (Xu et al. 2008). The breeding effort to exploit the other wild species for drought tolerance in tomato is limited. With the availability of genetic resources and genomic resources on a pan-genome scale, the efforts are likely to focus more on climate resilience in tomato.

1.7 Brief Account of Molecular Mapping of Tolerance Genes and QTLs

1.7.1 Genomic Studies on Abiotic Stress Tolerance in Tomato

Land plants due to their sessile nature have to endure the harsh environmental conditions. These harsh environmental conditions are collectively labelled as abiotic stresses. Abiotic stresses like high salinity, flood, drought, extreme heat and cold pose a great threat to agriculture (Wang 2003; He et al. 2018). Conventional breeding approach is time consuming, untargeted and hence laborious. Marker assisted breeding and transgenic approaches to obtain elite lines of plants are necessary to meet the ever growing global demands (Ahmar et al. 2020). *Solanum lycopersicum*, commonly known as tomato is sensitive to some abiotic stress like heat and drought but can show tolerance towards moderate levels of salinity (Ayenan et al. 2019; Ors et al. 2021). The breeding of the commercial tomato with the wild species, *Solanum pimpinellifolium* the larger of two genomes (811 Mb), will help immensely as it is a treasure chest for abiotic stress tolerance genes (Razali et al. 2018). Molecular markers can be used to differentiate between multiple varieties aiding in diversity analysis or identify QTLs that impart a specific trait in this case abiotic stress tolerance. In addition to that, transgenic plants can be created to better tolerate the various harsh environmental conditions. The various studies exploring these ideas in tomato related to abiotic stress tolerance are discussed below.

1.7.2 Genomic Diversity Analysis

Molecular and phenotypic evaluations offer an easy way to differentiate between species and the genetic divergence. The molecular evaluation provides deeper insights into the genetic structure whereas the phenotypic counterpart offers less than adequate variations for the intraspecific discrimination and is affected by environmental conditions (EL-Dijkhuizen et al. 1996; Mansy et al. 2021). Molecular markers can be used in diversity analysis with respect to specific characters by subjecting the plants to various stimuli. High temperature stress tolerance is one of the most widely researched topics and rightly so because of the threat of global warming due to climate change. The trends indicate that the average global temperature will rise from 1 °C to 3.7 °C from years 2081–2100 comparing 1986–2005. The increase in temperature will adversely affect tomato production. Even a rise of 1 °C in the average daily temperature can have disastrous impact on the plant's survival (Ayenan et al. 2019).

Six lines of tomato were chosen for the evaluation of heat stress tolerance and diversity between them was established with the help of various molecular markers. A total of 13 unique ISSR, RAPD and SCoT markers were identified in the study (EL-Mansy et al. 2021). Similar study involving 22 tomato accessions from different parts of India resulted in the identification of 10 SSR markers related to heat stress and three related to more than one trait (Amrutha et al. 2021). A study involving 15 tomato genotypes, including four local varieties of Turkey, 10 heat tolerant varieties obtained from Asian Vegetable Research and Development Center and one *S. pimpinellifolium*, were screened for heat tolerance. The SSR, SRAP markers were employed to identify the differences. Thirty six polymorphic SSR markers with 44.7% polymorphic bands, and 11 SRAP markers with 28 polymorphic bands were reported (Comlekcioglu et al. 2010). Eleven commercial tomato genotypes were analysed under heat stress using 20 RAPD, 15 ISSR markers, individually and together. They yielded, 25, 38 and 31.5 polymorphic bands in combination, respectively (Mansour et al. 2009).

Plants can have similar responses to drought, salinity and sodicity stresses, as it is a common phenomenon in the arid and semi-arid regions of the world. Both can result in the production of ROS which alter the normal cellular metabolism in plants (Dajic 2006; Uddin et al. 2016; Arif et al. 2020). High salinity conditions reduce the ability of plants to absorb water as a result of low water potential around root system. It leads to reduction in growth rate similar to drought stress effects. The combined effects of salinity and drought are more severe on plants than their occurrence individually (Dasgan et al. 2018; Ors et al. 2021). The response to salt and drought stress in plants involves osmotic and ionic signalling to re-establish cellular homeostasis, detoxification to repair the damage caused by stress and signal and co-ordinate cell division to achieve growth (Zhu 2003). Tomatoes are moderately sensitive to salinity but can be affected under severe drought conditions (Ors et al. 2021).

Gharsallah et al. (2016) studied three tomato varieties commonly grown in Tunisia under salt stress and identified 19 polymorphic SSR markers. They also used genotypic and phenotypic associations from multiple loci into a multi-layered network which can guide in the introgression of traits related to salinity stress. SNP markers can be used to identify variations in genes related to specific stress. Ninety four genotypes were subjected to SNP analysis with respect to four genes, *DREB1A*, *VP1.1*, *NHX1*, and *TIP*. The researchers identified 5 SNPs/InDels in two of the four genes (*DREB1A*, *VP1.1*) accounting for 17 to 25% of phenotypic variation related to salinity tolerance (Rao et al. 2015). Fifteen tomato cultivars were used to observe the diversity with respect to drought stress using ISSR markers by Metwali et al. (2016). Ten ISSR markers were identified with polymorphic ratio percentage ranging from 14.5 to 62.5%. The 15 genotypes showed clear cut genotypic differences and their potential are used in breeding programmes. Molecular markers used in various diversity analysis studies are presented in Table 1.4.

Table 1.4 Different molecular markers used in various diversity analysis related to different abiotic stress in tomato

Stress	Marker type	Number of Unique markers	Number of accessions/genotypes	References
Heat	ISSR, RAPD and SCoT	13	6	EL-Mansy et al. (2021)
	SSR	10	22	Amrutha et al. (2021)
	ISSR and SRAP	47	15	Comlekcioglu et al. (2010)
	ISSR and SRAP	35	11	Mansour et al. (2009)
Salinity	SSR	19	3	Gharsallah et al. (2016)
	SNP	5	94	Rao et al. (2015)
Drought	ISSR	10	15	Metwali et al. (2016)

1.8 Different Markers Used to Identify QTLs Related to Abiotic Stress Tolerance

The response towards abiotic stress at the gene level, can be non-specific as shown by Foolad et al. (2003a) when they discovered QTLs on chromosomes 1and 4 which was responsible for cold, drought and salinty stress tolerance. However, they discovered stress tolerance specific QTLs as well. The efforts of identifying QTLs related to abiotic stresses in tomatoes are summarized below.

1.8.1 Heat Stress

Several studies have been conducted based on molecular markers to identify QTLs related to heat stress tolerance in tomato. The use of an eight way MAGIC (Multi-Parental Advanced Generation Inter Cross) and a CC (Core Collection) population to identify QTLs related to heat stress was explored by Bineau et al. (2021). The 166 and 98 unique QTLs including 69 pQTLs (plasticity QTLs) related to heat stress tolerance in both the populations were identified. A study where a Recombinant Inbred Line (RIL) of a cross between *S. lycopersicum* cv. Money maker and *S. pimpinellifolium* (accession CGN14498) aided in the investigation of the effects of various environmental stresses with varied nutrient supplements (low nitrogen and high phosphate) and identification of 13 QTLs using a linkage map published in an earlier study (Geshnizjani et al. 2020; Kazmi et al. 2012). In another RIL of the same cross, Gonzalo et al. (2020) using the SNP SOLCAP Infinium chip designed by Sim et al. (2012) reported three newly identified QTLs on chromosomes 2, 3 and 4 in plants grown at higher temperatures (35 °C). It also must be noted that they reported 20 other QTLs related to fruit trait under heat stress in plants grown at lower temperatures (25 °C and 30 °C).

The use of meta-analysis to identify highly relevant QTLs was performed by Ayenan et al. (2019). They identified 13 mQTLs related to heat stress tolerance from studies published earlier (Grilli et al. 2007; Lin et al. 2010; Xu et al. 2017; Driedonks et al. 2018; Wen et al. 2019). Two SNPs associated with heat tolerance were identified which are linked to three known QTLs in the study by Ruggieri et al. (2019). One of the SNPs (solcap_snp_sl_33830) was present in the gene coding for cytochrome P450 and other the gene is yet to be identified. They also reported few other SNPs that are related to genes imparting high temperature tolerance. A combinatorial approach would result in identification of more relevant QTLs. Such an approach where QTL-Seq analysis and conventional mapping was done by Wen et al. (2019) resulted in the discovery of five consensus heat stress tolerance related QTLs with four candidate genes.

Through genotyping and identifying SNPs, Xu et al. (2017) identified 13 QTLs, one of which was linked to pollen viability in the F2 population of Nagcarlang and NCHS-1 cultivars of tomato. Driedonks et al. (2018) using heat tolerant *S. pimpinellifolium* with either a heat susceptible, *S. lycopersicum* cultivar Moneyberg (MB) or a heat susceptible *S. pimpinellifolium*, identified 13 QTLs related to heat stress tolerance. In another study, RAPD, ISSR and AFLP markers were used to identify 21 QTLs by Lin et al. (2010) where a cross between heat-tolerant breeding line, CL5915-93D4-1-0-3 (*Solanum esculentum*) and a heat-sensitive wild accession, L4422 (*S. pimpinellifolium*) were used. These QTLs were related to fruit characteristics, seed number and Brix value.

Yeh et al. (2006) have used the same markers to identify 6 QTL regions related to various traits linked to heat stress tolerance. Grilli et al. (2007) using Fluorescent Amplified fragment length polymorphism (FAFLP) identified 6 QTLs related to heat stress tolerance in a cross between Jab-95 (heat-tolerant) and cultivar Caribe (heat-susceptible). In a related note, a marker-trait analysis study conducted on 10 tomato genotypes was used to identify SNPs and InDels associated with key heat stress response genes. The genotype, E42 showed 129 polymorphic sequences derived from the stress tolerant wild type *S. pimpinellifolium* (Olivieri et al. 2020). The identified QTLs related to heat stress are presented in Table 1.5.

1.8.2 Salinity Stress and Drought Stress

Various studies have been conducted to understand the molecular mechanisms behind salt and drought stress tolerance and to identify molecular markers that differentiate the superior tolerant varieties in tomato. Grafting conferring tolerance against abiotic stresses is well established (Zijlstra et al. 1994; Schwarz et al. 2010; Colla et al. 2013; Meimandi et al. 2020; Singh et al. 2020). In a study conducted to identify the genetic differences between grafted and the un-grafted RIL of *Solanum pimpinellifolium* lines, Asins et al. (2021) identified 46 QTLs related to water-deficit stress tolerance

Table 1.5 List of identified QTLs and associated traits and chromosomal location related to heat stress tolerance in tomato

No. of QTLs	Marker	Chromosome(s)	Traits	References
69 (pQTLs)	SNP	1–12	Soluble solid content, pH, Number of fruits, Plant height, leaf length, Fruit set, Fruit weight, Fruit colour Stem diameter, Flowering time, No. of flowers	Bineau et al. (2021)
13	SNP	1,4,6, 10 and 11	-	Geshnizjani et al. (2020)
22	SNP	1, 2, 4, 6, and 12	Flower number, Fruit set, Fruit set proportion, Stigma exsertion, Pollen tube germination, Pollen viability	Gonzalo et al. (2020)
13 (mQTLs)	–	1, 2, 3, 9, 11 and 12	Pollen viability, Pollen number, Style protrusion, Anther length, Number of flowers per inflorescence, Inflorescence number, Relative electrical conductivity, Chlorophyll content, Fv/Fm	Ayenan et al. (2019)
5 (consensus QTLs)	SNP	1 and 2	Relative electrical conductivity, Chlorophyll content, F_v/F_m	Wen et al. (2019)
13	SNP	1, 2, 3, 4 7, 9 and 12	Pollen viability, Pollen number, Style protrusion, Style and Anther length	Driedonks et al. (2018)
13	SNP	1, 2, 3, 7, 8 and 11	Pollen viability, Pollen number, Style protrusion, Style and Anther length	Xu et al. (2017)
21	ISSR, RAPD and AFLP	–	Fruit weight, Fruit number, Brix value, Fruit setting, Seed number and Flower number	Lin et al. (2010)
6	ISSR, AFLP and RAPD	2, 3 and 4	Yield	Yeh et al. (2006)
6	FAFLP	–	Fruit set	Grilli et al. (2007)

with candidate genes including transcription factors having significantly enriched GO terms. These include genes involved in cell wall, root development, osmotic and hydraulic adjustments. In 2015, Asins and others identified 7 QTLs relating to transport of four macro and micro nutrients in leaf (K, B, Mg and Mo) and total solid contents in fruit in moderately salt-stressed 130 F_{10} grafted lines of the hybrid between a salt-sensitive genotype of *Solanum lycopersicum* var. Cerasiforme and a salt-tolerant line from *S. pimpinellifolium*.

Geshnizjani et al. (2020) identified 14 and 27 QTLs related to salt and drought stress tolerance, respectively using SNPs identified earlier by Kazmi et al. (2012). Studies by Diouf et al. (2018, 2020), revealed 46 DEGs (Differentially Expressed Genes) between drought-stressed and control tomato plants associated with35 QTLs previously identified in eight tomato genotypes related to drought stress. Another study into the effect of drought stress on cultivated tomato identified 11 QTLs of which two were labelled as interactive QTLs with genes related to water deficit stress (Albert et al. 2016). Nineteen drought stress related QTLs were identified on chromosome 9 of the NIL population of cross between *S. habrochaites* and *S. lycopersicum.* Most QTLs were identified on the centromeric end suggesting an area of valuable alleles belonging to the wild type (Lounsbery et al. 2016).

Two introgression lines (IL) of *Solanum pennellii* and *Solanum lycopersicoides* were used to identify six QTLs related to salt stress at seedling stage (Li et al. 2011). Estañ et al. (2009) reported eight QTLs related to fruit yield due to salinity tolerance from the wild type species in the F9 population of the cross between *Solanum lycopersicum* var. cerasiforme, as female parent, and two salt tolerant lines, as male parents, from *S. pimpinellifolium* and *S. cheesmaniae.* Villalta et al. (2007) also reported eight QTLs related to salinity tolerance from the F7 population of the same crosses.

Foolad et al. (2003b) identified four QTL regions specific to drought stress tolerance using RFLP markers in crosses between different accessions of *L. esculentum* and *L. pimpinellifolium.* Six QTL regions specific to salt stress using RFLP markers in a cross between *L. esculentum* and *L. pimpinellifolium* were identified and later in the same cross, the effect of salt stress in germination and vegetative stages of tomatoes revealed seven and six QTLS, respectively (Foolad et al. 1998a; 2001; Foolad 1999). The QTLs identified related to salt and drought stress tolerances are presented in Table 1.6.

1.8.3 Cold Stress

Cold stress or low temperature stress is alienated as chilling stress (<15 °C) and freezing stress (< 0 °C). After drought stress low temperature stress is most harmful to plants. Tropical plants adapt poorly to cold stress but temperate plants can tolerate freezing temperatures (Chinnusamy et al. 2007; Ritonga and Chen 2020). Generation of ROS due to cold stress can have devastating effects on plants (Rezaie et al. 2020). In tomato, cold temperatures slow down the ripening of tomato fruits. This occurs due to the changes in activity of the hormone ethylene and its response factors (Bergevin et al. 1993; Mata et al. 2019). Also, rapid root chilling in tomatoes impedes water movement from root to shoot having similar effects to water deficit stress (Arms et al. 2015).

When the relative germination rate and chilling index was used to identify QTLs in a population raised from the cross between *S. lycopersicum* and *S. pimpinellifolium*, nine QTLs related to cold stress tolerance were identified (Liu et al. 2016). Water

Table 1.6 List of identified QTLs, associated traits and chromosomal location related to salinity and drought stress tolerance in tomato

Type of abiotic stress	No. of QTL	Marker	Chromosome(s)	Traits	References
Drought stress	46	SNP	1–12	Shoot water content, Xylem sap (ABA, Mg, Mn, B, Zr, P)	Asins et al. (2021)
	27	SNP	1, 2, 3, 4, 5, 6, 8, 11 and 12	–	Geshnizjani et al. (2020)
	35	SNP	1–12 (except 5)	Time to flower, Fruit weight, Soluble solid content, Time to ripe, Fruit firmness, Leaf length	Diouf et al. (2018)
	56	SNP	2, 3, 4, 6, 7, 8, 9, 11, 12		Albert et al. (2016)
	19	SNP	9	Days to first green fruit and ripe fruit, Total fruit yield, Shoot dry weight, Ratio of ripe fruit to total yield, Leaf area, Phenolic content	Lounsbery et al. (2016)
	6	RFLP	1, 4, 8, 9 and 12	–	Foolad et al. (2003b)
Salinity stress	14	SNP	4, 5, 6, 7, 8 and 10	–	Geshnizjani et al. (2020)
	7	SNP	1–12	Soluble solid content, Citric acid, Leaf dry weight, Fruit weight, Leaf fresh weight, Macro and micro nutrients	Asins et al. (2015)
	6	RFLP	4, 6, 9 and 12	–	Li et al. (2011)
	8	SSR	3, 5, 6, 9 and 11	Total and ripe fruit weight, Number of days to flowering, Number of fruits,	Estañ et al. (2009)
	8	SSR	1, 3, 5, 6, 7, 11 and 12	Dry leaf and stem weight, Leaf area, Na and K content in stem and leaves, Total Na content	Villalta et al. (2007)
	6	RFLP	1, 2, 5, 7 and 12	–	Foolad et al. (1998a)
	7 and 6	RFLP	1, 2, 3, 5, 7, 6 and 9, 11 and 12	–	Foolad (1999); Foolad et al. (2001)

Table 1.7 List of identified QTLs, associated traits and chromosomal location related to low temperature stress tolerance in tomato

No. of QTLs	Marker	Chromosome(s)	Traits	References
9	SSR	1, 2, 3, 4, 9 and 12	Relative germination rate, Chilling index	Liu et al. (2016)
7	RFLP	1, 5, 6, 7, 11 and 12	2 h cold stress, 6 h recovery post cold stress	Truco et al. (2000)
3–5 putative QTLs	RFLP	1 and 4	–	Foolad et al. (1998b)

deficit due to root chilling has been studied by Arms et al. (2015) and have performed a high resolution mapping of a region in chromosome 9 named *stm9*, initially identified by Truco et al. (2000) with 22 putative genes. Truco et al. (2000) have also identified several QTLs related to chilling stress using RFLP markers. After two hours under cold stress, three QTLs were responsible for wilting with a QTL on chromosome 6 having a negative effect. In the recovery after six hours, it is reported that four QTLs were responsible. The MAPMAKER/QTL and QGENE software were used to identify 3–5 cold stress related QTLs in a cross between cold sensitive *L. esculentum* and *L. pimpinellifolium* (Foolad et al. 1998b). In tomato many QTLs related to a specific stress tolerance are identified and this makes understanding the performance of tomato under harsh environmental conditions much easier. The QTLs related to low temperature stress are listed in Table 1.7.

1.9 Genes Orchestrating Abiotic Stress Tolerance in Tomato and Transgenic Efforts

Discovering the genomic regions that are involved in abiotic stress tolerance is necessary to develop and select lines that are better at withstanding harsh environmental conditions. Understanding the regions imparting stress tolerance requires the identification of the genes pulling the strings. The key genes imparting abiotic stress tolerance code for regulatory proteins like transcription factors, functional proteins and other proteins for the protection of biomolecules that are necessary for the survival of the cell and plant as a whole (Agarwal et al. 2006). Studies on the transcriptome of plants under stress reveals key genes by highlighting the DEGs that play a role in various abiotic stress tolerance (Weiss and Egea-Cortines 2009; Bita et al. 2011; Chen et al. 2015a, b; Cruz-Mendívil et al. 2015; Fragkostefanakis et al. 2016; Liu et al. 2017; Zhang et al. 2017b; Bouzroud et al. 2018; Mu et al. 2021b).

Transcription factor families like MYB, AP2/ERF, NAC, WRKY, bZIP have been reported to regulate abiotic stress response in tomato (Yánez et al. 2009; Hsieh et al. 2010; Sharma et al. 2010; Cao et al. 2013; Zhu et al. 2014; Hichiri et al. 2017; Klay et al. 2018; Gao et al. 2020; Zhu et al. 2020). Since, transcription factors regulate gene expression, understanding their roles and the genes they regulate is necessary. The MAPK pathway gets triggered under abiotic stress. Genes like *SlMPK*1, 2 and3 are involved in response to heat and oxidative stress (Nie et al. 2012; Li et al. 2014). Calcium mediated signaling is carried out by calcium binding proteins which, in turn binds to the specific CAMTA transcription factor in the family. In case of elevated salt levels in the environment, the SOS salt response pathway comes in to effect. One of the proteins SOS3 (calcium binding protein), senses the calcium levels in the cell and activates SOS2 (Serine /Threonine kinase) and through SOS1 (Na^{+}/H^{+} transporter) the excess salt is transported out of the cell (Ishitani et al. 2000; Huertas et al. 2012; Noman et al. 2021). Production of ROS is necessary under abiotic stress since it is plays role along with hormones and other signaling mechanisms in responding to stress (Devireddy et al. 2021). The ROS formed under various stresses has to be scavenged since it can have harmful effects on the cell. Enzymes like SOD, CAT, ascorbate peroxidase (*APX*), dehydro ascorbate reductase (*DHAR*), glutathione reductase (*GR*), mono dehydro ascorbate reductase (*MDHAR*) and guaiacol peroxidase (*GPX*) help in scavenging the formed ROS before it causes irreversible damage (Das and Roychoudhury 2014). A transcription factor *SlGRAS10* when down regulated resulted in elevated levels of the scavenging enzymes and ultimately reduced the levels of ROS (Krishna et al. 2019; Habib et al. 2021). Recent efforts on transgenic approach towards tolerance towards abiotic stress are highlighted in Table 1.8.

Once the roles of genes are understood, the logical next step is to check the effects of overexpressing or silencing said genes. It can also be used to understand the function of the gene as well. Apart from that, expression or overexpression of foreign proteins that have superior properties to their native counterparts have been attempted. These studies give us insights in to the various genes that can be used to impart a specific stress tolerance.

1.10 Sol Genomics

It is used to store genetic and genomic information, for Solanaceae species, such as tomato, potato, tobacco, pepper, eggplant and petunia. It is a community driven, genome sequence based database where users can update and delete sequence related information. It functions as a model organism database (MOD) but it is community curated (Fernandez-Pozo et al. 2015). The sequencing of a high quality reference resulted in mapping sequences of other genomes on to the corner stone sequence (Mueller et al. 2005). It has various tools related to sequencing, mapping and two tomato specific tools i.e., Tomato Expression Atlas and Tomato Expression Database. It contains information on the various wild type species related to tomato and that can be helpful for introgression of abiotic stress traits into the commercial varieties. The genes that are annotated, including the abiotic stress related genes can be helpful in analysing one's transcriptome data.

SolCyc, a set of Pathway/Genome Databases (PGDB) provide information on the metabolic pathways and enzymatic reactions for the Solanaceae species. The Sol

Table 1.8 List of transgenes and effects related to different abiotic stress tolerance

Type of stress	Modification	Effects	References
Drought stress	Overexpression of *SlNAC6*	Delay in growth; reduced water loss and oxidative damage	Jian et al. (2021)
	Co-overexpression of *AtDREB1A* and *BcZAT12*	Enhanced drought tolerance; reduced electrolyte leakage, H_2O_2 and elevated level of relative water content, chlorophyll colour index	Krishna et al. (2021)
	Overexpression of *SlGATA17*	Better drought tolerance through regulating the activity of the PAL gene of the phenyl propanoid pathway	Zhao et al. (2021)
	Overexpression of *SlGRAS4*	Enhanced drought stress tolerance and upregulation of genes for ROS scavenging enzymes due to interaction with positive regulators of ABA signalling	Liu et al. (2021)
	Silencing of *SLB3*	Decreased drought tolerance and decreased levels of SOD enzyme, increased ROS, proline and peroxidase enzyme	Wang et al. (2020c)
	Crispr cas9 mediated *SlMAPK3* knockout	more severe wilting symptom, higher hydrogen peroxide content, lower antioxidant enzymes activities, and suffered more membrane damage under drought stress. Up- or down-regulated expressions of drought stress-responsive genes including *SlLOX*, *SlGST*, and *SlDREB*	Wang et al. (2017)
Salinity stress	Overexpression of sly-miR398b	Decreased plant growth and reduced biomass; downregulation of scavenging enzymes and consequent increase in O_2 radicals	He et al. (2021)
	Overexpression of *SlBZR1*	Reduced plant growth, delayed flowering, smaller and curly leaves; upregulation of stress related genes	Jia et al. (2021)
	Overexpression of *LeNHX4*	Increased fruit number and size under salinity and normal conditions; increased tolerance to salinity stress	Maach et al. (2020)
	Co-overexpression of *LeNHX2* and *SlSOS2*	Increased tolerance to salinity, increased yield, biomass, proline levels and total soluble solids in fruit	Maach et al. (2021)
	Overexpression of *SlCOMT1*	Improved salt stress tolerance by altering melatonin levels, higher levels anti-oxidant enzyme activity and higher ascorbic acid (AsA) and glutamate (GSH) accumulation levels	Sun et al. (2020)
	Crispr Cas9 mediated partial excision of *SlHyPRP1*	Various excised motifs of the *SlHyPRP1*exhibited varied results with respect to growth and germination with most being better than the WT	Tran et al. (2021)

(continued)

Table 1.8 (continued)

Type of stress	Modification	Effects	References
Heat stress	Overexpression of *HsfB1*	Overexpression leads to the accumulation of phenols, flavonoids due to upregulation of genes involved and knock down leads to accumulation of polyamine putrescine, glucose and sucrose	Paupière et al. (2020)
	Overexpression of *SlWHY1*	Upregulation of *SlHSP21.5A* leading to increased membrane stability, soluble sugar content and reduced ROS contributing to heat tolerance	Zhuang et al. (2020)
	Overexpression of *SlSNAT*	Increased levels of melatonin and thermotolerance and interaction with HSP40 protect the SNAT enzyme	Wang et al. (2020b)
Cold stress	Overexpression of *SiFBA5*(*Saussurea involucrata*)	Increased cold tolerance, malondialdehyde, CAT, SOD and POD production and improved photosynthetic efficiency due to increased FBA expression in chloroplasts and promoted Rubisco expression	Mu et al. (2021a)
	Overexpression of *BoCRP1* (*Brassica oleracea*)	Increased tolerance to chilling stress; overall improved rate of seed germination, increased accumulation of osmoprotectantsand increased root length; reduced membrane damage	Wani et al. (2021)
	Overexpression of *SlHY5*	Better cold tolerance; upregulation of genes related to antioxidant enzymes like SOD and CAT, anthocyanin biosynthesis genes *CHS*, *CHI*, and *F3H* and cold induced genes *PR1, CYSb, LEA*, *Osmotin*, and *ICE1*	Han et al. (2020)

genomics website has a genomic selection tool that relates the genotype and phenotype data to predict the phenotype from genotypic data. Apart from the genotype related data and tools, it offers phenotype database as well (Fernandez-Pozo et al. 2015).

1.11 Conclusion

Tomato being nutritionally an important crop is grown worldwide in diverse geographical regions. Though, better crop management practices and cultivars are available for realizing higher yield, tomato crop is affected by various abiotic stresses during various phenophases. Under climate change conditions the occurrence of these abiotic stresses are likely to increase and cause damage to the production, productivity and quality. In the coming years due to demand from the growing population, there is a need to enhance the production and sustain productivity and quality. The abiotic stresses may occur either individually or in combination causing adverse effects on the crop. Tomato is sensitive to high temperature, salinity, cold, deficit, and excess moisture stress conditions. Hence, better understanding with respect to molecular, physiological, biochemical and morphological changes occurring due to impact of various abiotic stresses is essential. The literature is replete with information on influence of abiotic stresses at different organizational level of tomato plant. Good information is available on tolerant sources both from cultivated and wild species. However, their exploitation in developing tolerant cultivars employing breeding methods is very limited.

The modern molecular tools employed in identification of QTLs, MAS, genome editing, gene discovery, transgenic approaches have facilitated better understanding of the response of tomato and its wild species with respect to various abiotic stresses. In terms of crop improvement there is reasonable understanding about various sources and traits imparting salinity stress, drought, flooding and other abiotic stresses. We have reasonable knowledge on root traits imparting tolerance to heat, drought, salinity and cold stresses. Knowledge of genomic diversity, genes involved and QTLs associated traits and chromosome location with respect to drought, heat, salinity and cold stresses are being generated and would be used in improvement programmes.

The approach of mining various genotypes with superior qualities for abiotic stress tolerance as well as differentiate them, using ideal, rapid and accurate techniques. The identified QTLs and markers, will aid in breeding programs aimed at producing abiotic stress tolerant lines of tomato. RNA Seq analysis and production of transgenic lines with foreign gene and/or overexpressing or silencing of native genes will help in gaining insights about the genes involved in abiotic stress tolerance. Recently genome editing technology has improved our ability to manipulate the tomato genome. The Sol genomics database is the latest tool with its ease of use and relevant information available for researchers', disposal to further the innovations and improvement of abiotic stress tolerance in tomato.

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Chapter 2
Genomic Designing for Abiotic Stress Tolerant in Potato

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Abstract Potato is an important food crop of the world. Besides other factors, potato crop suffers from various abiotic stresses such as heat, drought, salinity, nutrients and frost. Of these, heat is the most important followed by drought stress for climate change point of view, while improving nutrient use efficiency in also essential to save environment and reduce cost of production. Salinity is another problem under irrigated condition, while frost is an issue of temperate climate. Overall, these environmental factors affect crop growth and ultimately reduce tuber yield. Potato is rich in diverse gene pool, however, a little has been utilized for its genetic improvement. With the increasing genomics resources like potato genome sequences, numerous functional genomics sequence data at multiple time points of various plant tissues, high-density potato maps and molecular markers particularly single nucleotide polymorphism (SNP) linked to the trait of interest, there is possibility of rapid potato breeding for tolerance to above abiotic stresses. This chapter highlights on the abiotic stress related information in potato on the genetic resources, genetic diversity, classical genetics and breeding, molecular mapping (linkage and association), gene cloning, marker assisted selection, genome sequence, genomics-assisted breeding, genomic selection, functional genomics, genome editing, nanotechnology, bioinformatics and social concerns.

Keywords Abiotic stress · Cold · Drought · Genomics · Heat · Molecular breeding · Potato · Salinity

2.1 Introduction

Potato (*Solanum tuberosum* L.) is the third most important human food in the world after rice and wheat. Potato has high yield potential, high nutritive values and is wholesome food (Chakrabarti et al. 2017). In addition to various other growth affecting factors under a climate change scenario, potato suffers from many

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abiotic stresses such as heat, drought, salinity, frost and nutrients (Dahal et al. 2019; Handayani et al. 2019). Cultivated potato crop requires moderate temperatures (about 18–20 °C) for tuberization, while nearly 25 °C is optimum for vegetative growth. Potato cultivation ranges from low to high altitudes and temperate to tropical/subtropical climates with moderate temperatures. In tropical regions having high temperature, potato yields are lesser than temperate or subtropical regions. Although, biotic stresses and poor cultural practices are major factors of low yield in tropical areas, yet abiotic environmental factors like high temperatures combined with limited soil moisture are also important factors. With the rising in global temperature under climate change scenario, heat stress in potato is the most important. Although, potato is an irrigated crop, drought stress is another problem where water is limiting especially African countries. Salinity is problem in high saline soil area, whereas frost is an issue in temperate climates. Moreover, improving nutrient use efficiency of potato is important particularly N fertilizers to save the environment and reduce production cost. Owing to its high productivity, short duration crop and nutritionally superiority, it has the potential to significantly augment the food availability in tropical regions. Variability exists in the potato germplasm and related species for these abiotic stresses. Therefore, top priority is required to be directed for introducing tolerance to abiotic stresses for expansion of potato cultivation in non-traditional areas of tropical and subtropical regions of the world (Demirel et al. 2020). Classical breeding has achieved a lot while developing abiotic stress tolerant varieties but implication of genomics is still limited in potato where genome sequence is available. This chapter highlights genomic designing of potato for abiotic stress tolerance.

2.2 Abiotic Stresses

2.2.1 Heat Stress

Potato is considered primarily a crop for cool and temperate climates. High temperature inhibits crop yield by overall reduction of plant development due to heat stress or by reduced partitioning of assimilates to tubers (Singh et al. 2015). Minimum night temperature plays a crucial role during tuberization in potato and largely determines whether plants will tuberize or not. Tuberization is reduced at night temperatures above 20 °C with complete inhibition of tuberization above 25 °C. The most important effect of high night temperature is on the partitioning of assimilated carbon between leaves and tubers. Exposure of potato plants to high temperature alters the hormonal balance in the plants. As a result most of assimilated carbon is partitioned to the shoots (stems and leaves) and not to the tubers. Heat stress tolerance breeding programs should consider the ability to tuberize at higher night temperature (above 22 °C), low shoot to root ratio at high temperature, and early crop maturity (short duration). Figure 2.1 shows response of plants to high temperature stress in potato.

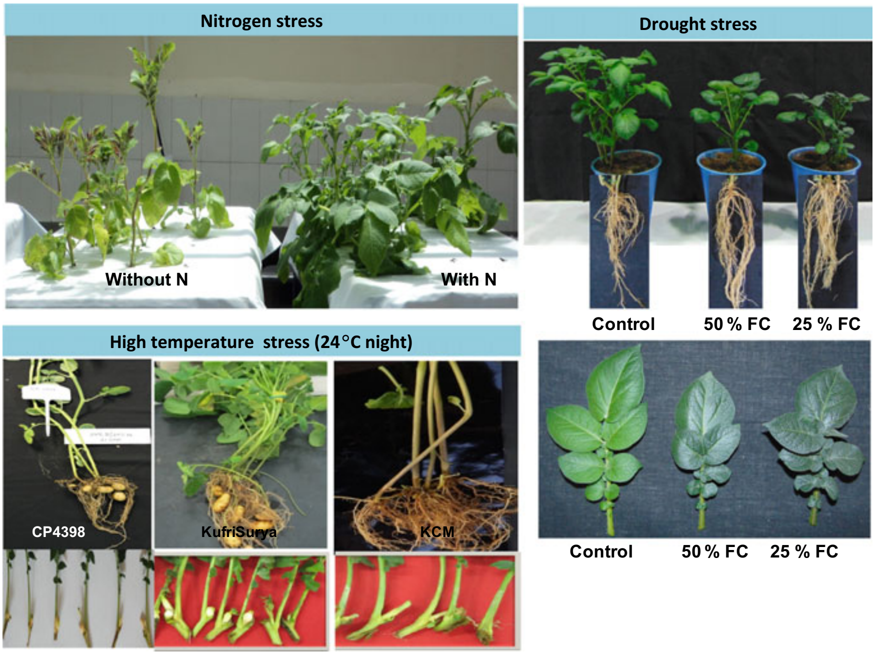

Fig. 2.1 Response of potato plants to different stresses such as nitrogen, high night temperature, and drought under controlled conditions. (FC: Field Capacity; cv. KCM: Kufri Chandramukhi)

2.2.2 *Drought Stress*

Potato is mostly an irrigated crop in plains and rainfed in hilly regions. Owing to shallow root system it is more prone to drought stress. Drought is an emerging problem of potato production due to availability of irrigation water in the world, which affects tuber yield as well as quality (Monneveux et al. 2013). Drought may occur due to erratic rainfall, inadequate irrigation and lack of water supply. Even with good irrigation water supply, moisture stress may occur because of high transpiration rates especially during high temperature in noon when root system cannot completely meet the water requirements of the plant. Drought may affect potato growth and production by reducing foliage biomass, decreasing photosynthesis rate per unit leaf area, and early crop maturity and shorter vegetative growth period. All stages of crop growth are sensitive to drought. Insufficient water supply in the period between emergence and the beginning of tuber bulking may therefore lead to a reduced growth rate of foliage and ground cover resulting in incomplete plant growth and yields below optimum. Potato plant is highly sensitive to water stress and the decline in photosynthesis is fast and substantial even at relatively low water potentials of –3 to –5 bars. Plants respond to water stress by closing their stomata thus shutting out the supply of CO_2. Tuber traits such as shape, cracking, dry matter content and reducing sugars are highly influenced by the availability of soil moisture during the vegetative period. Taking into account production conditions and the present yield levels, it is estimated that the average potato yield in the world could be increased by at least 50% if the water supply to the crop could be optimized. Therefore, breeding of drought stress tolerance is now a priority in potato applying genomics approaches. This is important while saving irrigation water to ensure yield and food security under the climate change scenario and growing demand of water. Figure 2.1 shows response of plants to drought stress in potato.

2.2.3 Nutrient Stress and Salinity

Major and minor nutrients are essential for good vegetative growth, yield and quality of potato (Handayani et al. 2019). Among all, nitrogen is the most important nutrient for plant growth and development including potato. Potato is a resource intensive crop and requires high N fertilizer (150–240 kg N/ha) to produce tuber yields (30–50 t/ha) in India. Despite the high cost of N fertilizer, potato crop uptakes nearly 50% of the total N applied and excess lost in the environment. Potato is a shallow rooted crop and mostly irrigated cultivations are practiced on the sandy-loam soils with excessive application of N fertilizers. This practice increases the chance of nitrate leaching, contamination to the groundwater and high cost of cultivation. In addition, improving P and K use efficiency of plants would also save additional costs. Though, field-based approaches have been applied in potato for improving nutrient use efficiency (NUE) for successful tuber production. However, improving N use efficiency of plant is one of the key options to minimize N losses, save the cost of production and improve the environmental quality to achieve sustainable crop yield (Tiwari et al. 2018c, 2020d). Most studies so far focused on agronomic managements for improving NUE in potato. Figure 2.1 shows response of plants to nitrogen stress in potato under aeroponics. In addition, salinity is another issue in highly irrigated conditions. Salinity could be either due to salinity of the soil or irrigation water applied to the crops. Salinity causes nutritional imbalances, restricts plant growth and development, early senescence and severely reduces tuber yield in the semi-arid and arid regions. Variability for salinity stress tolerance exists in potato germplasm, which can be harnessed for development of varieties (Ahmed et al. 2020).

2.2.4 Frost Stress

Frost is the major problem of temperate growing regions of potato. Temperatures below −2 °C can result into partial or complete loss of the crop. In temperate zones, frosts can occur during spring season when the crop is at initial stage of vegetative growth or during autumn when it is near to maturity. Higher crop losses occur in tropical highlands and subtropical plains where frosts can occur any time during the crop growth period. In India more than 80% of potato is grown during winter in subtropical plains and the crop is prone to frosts during the months of December and January. Based on the field observations, two types of frosts are often distinguished namely ‘White frost’ that occurs when there is a decrease in temperature and high relative humidity, and ‘Black frost’ that occurs under low temperatures and much drier conditions, hence more damaging and severe because plant tissue is darkened immediately. Acclimation or hardening may increase the resistance to frosts in many plants. Exposure of the plants to prolonged low temperature is effective in increasing resistance to frost injury in wild potato species.

2.3 Genetic Resources

Abiotic stresses like heat, drought, nutrients, salinity and frost have significant impact on potato production affecting yield, tuber quality and marketable value. Cultivated potato is autotetraploid with highly heterozygous and a narrow genetic base. The genetic base of the cultivated potato can be expanded by introgressing useful genes from wild *Solanum* species for abiotic stress and others. *Solanum* species evolved under a range of different climatic conditions and they can be found between sea level and 4500 m altitude in temperate environments, humid tropical climates and even in deserts (Hawkes 1990). *Solanum* species offer a vast diversity of traits for breeding. The use of wild species has been suggested as a means to increase tolerance to many stresses including abiotic and thereby improve productivity in a range of crops. Currently large amounts of potato germplasm containing useful alleles are available in gene banks around the world. However, during its evolution and adaptation, potato passed through a genetic bottleneck resulting in a relatively narrow genetic base in the present varieties. Efforts have been made to broaden the genetic base over a century and but still efforts are required to improve abiotic stress tolerance.

Bradeen and Haynes (2011) proposed a potato gene pool concept based on endosperm balance number (EBN), where primary gene pool (5%, 5 species) species are cultivated potato which are 4X (4 EBN), the secondary gene pool (68%, 58 species) is the largest and contains wild relatives which are easy to cross to cultivated potato directly or after ploidy manipulations, and tertiary gene pool (27%, 23 species) species have 1 EBN. These species do not cross directly to cultivated potato but may be introgressed through bridge crosses, embryo rescue or somatic fusion. The potato germplasm resources is composed of 117 wild relatives, four land races, cultivated species and modern cultivars (Spooner et al. 2014). Wild potatoes are distributed from the south-western United States southward to central Chile and Argentina (Spooner et al. 2014). Species richness is greatest in central Mexico and in the Andean highlands. Wild potato species are found in highly diverse habitats including cloud forests, cactus deserts, scrub vegetation, mountain pastures, high grasslands and pine forests. They carry genes for traits that have not been identified in cultivated potato. Designing of crops adapted to unpredictable climatic changes like drought, heat, nutrients, salinity and frost need utilization of available genetic resources. This will facilitate effective use and pre-breeding for introgression of desired genes from wild species, which have evolved resilience against adverse climatic condition over centuries. In addition to various techniques to utilize wild resources, somatic hybridization has been used widely for introgression of genes from wild species into cultivated potato (Tiwari et al. 2018b). Further, this will help easy access to desired variability, both genetic and allelic for their use in further genetic improvement or designing of cultivars suited to changing climatic conditions.

2.4 Classical Genetics and Traditional Breeding

Earlier potato breeding has mainly focused on yield, quality and biotic stress tolerance, and less attention was driven to breed for abiotic stress tolerance. Present variation in the potato gene pool can be exploited to breed cultivars with tolerance to abiotic stress. There are a large number of potato traits that are regulated by multiple genes, or are quantitative in nature. Most important physiological and agronomic traits are under the control of a very large number of genes, each of which may have relatively low individual effect. The desire to obtain genetic gain in such highly complex and low heritability traits has led some breeders to use progeny tests to identify superior parental combinations. Progeny tests will help to determine the breeding value of the parent for these traits. Phenotypic selection of individuals for highly complex traits has been shown to be less effective than the use of progeny tests for family selection. Progeny testing has been extended to a range of traits in potato breeding (Bradshaw et al. 2009).

Conventional breeding strategies are too slow because they are essentially based on phenotypic selection, involving crosses between tetraploid varieties and advance clones, and then field evaluation and selection. The inherent genetic complexity of potato has made breeding time consuming and often with unpredictable results. Quantitative characters are difficult to maintain intact during breeding process and hence identification of the highly heritable sources and robust screening procedures is important. The selection cycle, from initial crosses to varietal release requires approximately 10 years or sometimes more than 30 years. Moreover, a major obstacle in using wild species in breeding is number of backcrosses required. Three to seven backcrosses are required to transfer a major dominant gene, which may take several generations to reconstitute phenotype of the commercial cultivar (Bradshaw et al. 2006). Erratic nature of the environmental factors and mechanisms involved in stress tolerance make the breeding program difficult. Most of the abiotic stress tolerance traits are polygenic in nature and strongly influenced by the genotype and environment interactions. These traits have low heritability and selection of such traits for tolerance will be less reliable. Careful analysis of environmental constraints together with genetic potential and trait combinations for yield, and simulation modelling are probably the best avenues to improve crop performance. Hence, biotechnology based potato breeding is required.

Efficiency and precision in plant breeding can be enhanced by use of diagnostic DNA-based markers and has been applied to potato breeding. Numerous genetic mapping experiments have been performed using potato and have identified DNA based markers linked to genes/quantitative trait loci (QTLs) for quality traits and agronomically important traits (D'hoop et al. 2008, 2014). The genome sequence of potato has been completed (844 Mb) and it revealed 39,031 protein coding genes in potato (Potato Genome Sequencing Consortium 2011). This information will be a platform for genetic improvement of potato. Genomic tools like high throughput resequencing, could be used to identify rare alleles and recessive alleles which are gone unnoticed till now and virtually inaccessible to potato breeders. Genotyping by sequencing combined with haplotype characterization has been shown to be an efficient method to characterize allelic variants that influence traits. When valuable alleles are identified and introgressed through hybridization, dense marker data can be used subsequently to efficiently retain the desirable alleles while removing the remaining donor genome. This process will be more effective in diploid inbred germplasm than in heterozygous tetraploids. In the genomics era, germplasm enhancement will increasingly be focused on identifying and introgressing alleles rather than cumbersome phenotyping traits. Alleles will come from a broad pool of genetic resources that includes wild species relatives of potato, landraces, cultivated potato itself, and distantly-related species. Genomics tools will greatly increase the efficiency of introgressing multi-genic traits and will make it possible to identify rare alleles and utilize recessive alleles. Introgression may occur through sexual hybridization, or molecular manipulations, but evaluation of progeny will increasingly involve assessing allelic composition and distribution of parental genomes.

The effect of abiotic stress can be observed visually and such variables can assist in development of cultivars to abiotic stress. To develop heat tolerant crop, leaves related traits like photosynthetic efficiency, haulm growth, tuber initiation and photosynthetic partitioning should be considered. Root architectural traits like root depth, high number of stolon roots, root mass are positively related with drought tolerance. Water use efficiency is another important trait which is correlated with drought stress. Cultivars having tolerance to single abiotic stress have been identified and developed. In India, recently a heat tolerant cultivar Kufri Lima has been released and Kufri Surya is another heat tolerant variety. In Japan, conventional breeding by crossing two commercial potato cultivars, Irish Cobbler and Konafubuki resulted in drought tolerant cultivar, Konyu (Iwama 2008) using high root dry weight as a selection criterion.

2.5 Diversity Analysis

Crop improvement primarily relies on examining the available genetic diversity to identify potential candidate germplasm for further use in research. Cultivars grown in extreme environments have always served as sources of novel genes/alleles/QTLs for improving tolerance traits in cultivated varieties. Screening of several *Solanum* species identified *S. juzepczuckii* and *S. curtilobum* as salt-tolerant (Silva et al. 2001), and three species, *S. juzepczuckii*, *S. acaule* and *S. curtilobum* are frost-resistant

(Martinez et al. 1996). The Andean potato landraces cultivated in cold and dry climatic conditions served as ideal candidates for identifying genes conferring tolerance to drought stress (Ritter et al. 2008; Vasquez-Robinet et al. 2008). Similarly, wild potato germplasm have also been useful in improving drought tolerance traits. The differential response of cultivated as well as wild varieties to salinity stress has identified crucial genes and pathways responsible for salt tolerance (Levy and Veilleux 2007) and water stress (Levy et al. 2013). Genetic diversity reveals wide variability in potato varieties and wild species (Tiwari et al. 2018a, 2019) and exploited in improvement programs.

Primitive forms of cultivated potato and their wild relatives provide rich, unique and diverse sources of genetic variation which could be a source of various traits for potato breeding. This may be because the habitat of these species is highly variable growing at different altitudes from sea level to over 4000 m in an extensive range of temperature, photoperiod, soil and water supplies (Hawkes 1990). Also, the wild genotypes adapted to different ecological conditions ranging from highland, tropics to desert regions. They are equally diverse in morphological traits too. Andes, the center of origin and diversity of the potato represents vast genetic resources. The harsh climate of the Andes is home to the valuable sources of germplasm which can withstand high light, drought and cold stress. They are recognized to be valuable in breeding programs for abiotic stress, environmental tolerance, processing and agronomic traits. The International Potato Centre (CIP), Lima Peru maintains over 600 clones of potato landraces which are found to be variable for agronomically important traits (Cabello et al. 2012).

In Andes, wild relatives are grown near farmers' fields and insect aided cross pollination between tetraploid and wild species is common. Grun (1990) described Andean potato as 'Genetic sponge' as it is capable of absorbing the genes from wild and cultivated relatives from its surroundings and hence cultivated potato is rich in allelic diversity at the centre of origin. So germplasm enhancement programs will help in improvement of the traits like disease resistance, abiotic stress, quality traits and agronomic traits under suboptimal conditions. Unlike disease resistance mechanism of species-specific nature, abiotic stress mechanisms are shared among the species. Hence germplasm enhancement for the abiotic stress mechanisms should be concentrated both in wild species and distantly related plants. Such traits can be introduced to potato through molecular biology approaches viz., transgenics and genome editing tools. Therefore, search in potato germplasm for genes that contribute to salt, drought, heat and cold tolerance in other Solanaceous crop like tomato will help in potato breeding programs.

2.6 Molecular Mapping of Tolerance Genes and QTLs

2.6.1 Linkage Mapping

The cultivated potato (*S. tuberosum* L. ssp. *tuberosum.*) varieties are tetraploid ($2n = 4x = 48$). The complex genetic inheritance (tetrasomic) and high heterozygosity of potato complicate its genetic mapping and therefore diploids were used in the most mapping studies. However, self-incompatability nature of the diploids prevents development of pure lines. Therefore, a number of common mapping approaches based on homozygous lines cannot be applied in potato unlike in other crops. The first potato genetic map was reported in 1988 using tomato RFLP (restriction fragment length poylmorphism) markers (Bonierbale et al. 1988). Two linkage maps were obtained from a cross between a diploid clone of *S. tuberosum* group Phureja and a diploid hybrid line from *S. tuberosum* group Tuberosum × (*S. tuberosum* group Phureja × *S. chacoense*). The alignment of the RFLP loci shows a high level of similarity to the tomato map and the major differences were paracentric inversions on three chromosomes. With advances in molecular markers, many maps have been constructed and used for identifying specific loci, and markers have been used in potato breeding for specific traits.

Simple sequence repeats (SSRs) markers have assisted progress in mapping the potato genome and are valuable for fingerprinting closely related genotypes (Tiwari et al. 2018a, 2019). Amplified fragment length polymorphism (AFLP) markers showed greater merit than multilocus SSRs for fingerprinting in a comparative study of RAPD (random amplified polymporphic DNA), ISSR (inter simple sequence repeat), AFLP, and SSR. These can be made from the expressed sequence tag (EST) database and made more systematical by potato genome information. With further advancement of genome research in plants, many relevant technologies can be applied to potato. DArT (diversity array technology) (Wenzl et al. 2004) benefits potato genetics. Finally, new sets of markers have been applied with advances in the potato genome sequencing. Single nucleotide polymorphism (SNP) has been used widely for marker generation in many species and many markers have been generated also in potatoes. Many quantitative trait loci (QTLs) for yield, agronomic and quality traits have been identified in potato (Bradshaw et al. 2008). The QTL mapping study for the abiotic stress tolerance is at infant stage. Quite a small number of QTLs have been identified for drought stress tolerance (Anithakumari et al. 2012).

Bacterial artificial chromosome (BAC) libraries have become the main vehicle for performing map-based gene cloning and physical mapping in potato. Several BAC libraries were constructed such as cultivated potato and wild species like *S. bulbocastanum* (Song et al. 2000). These libraries represent a potentially useful resource for the study of comparative genome organization and evolution in potato and other Solanaceous crops. A BAC library was used to make the ultra-high-density (UHD) genetic and physical map of potato with 10,000 AFLP loci (van Os et al. 2006). In addition, BAC libraries were used for fluorescence in situ hybridization

(FISH) to develop chromosome-specific cytogenetic DNA markers for chromosome identification in potato (Dong et al. 2000).

Drought tolerance was surveyed by various physiological parameters such as relative water content, stomatal conductance and chlorophyll fluorescence measurements (Schafleitner et al. 2007). However, these traits show less level of heritability, are controlled by several genes and their epistatic in nature restricts the breeding in potato for drought tolerance. Anithakumari et al. (2011) have identified 23 QTLs (13 QTLs under well-watered conditions, 7 under drought stress condition and 3 recovery QTLs) in a diploid mapping population, and the genes underlying these QTLs were related to root to shoot ratio, plant height, shoot fresh weight, shoot dry weight, fresh root weight, root dry weight, root length, fresh biomass and dry biomass. Interestingly, the study also found the co-localization of SNPs with root to shoot ratio QTL, thus proposing their applicability in MAS for drought tolerance in potato. QTLs associated with root length allow the selection of plants with desirable root characteristics in a non-invasive method as root traits are of immense importance in tolerating drought. In addition, QTLs associated with carbon radioisotope discrimination, chlorophyll content and chlorophyll fluorescence were also identified (Anithakumari et al. 2012), which can serve as good selection criteria since they are easy to measure, fast, and allow little or no sample destruction. Recently, Sharma et al. (2013) have constructed a dense genetic and physical map for diploid backcross progeny of potato using 2469 markers, including SSR, diversity array technology (DArT), and SNPs, and using the same genotypic data of these markers. Khan et al. (2015) constructed maternal and paternal maps to carry out the first QTL study for drought tolerance. The study identified 45 genomic regions associated with nine traits in well-watered and terminal drought treatments and 26 QTLs associated with drought stress. These QTLs will promisingly be used in the breeding of potato for durable tolerance to drought, using conventional as well as genomics-assisted breeding approaches.

2.6.2 Association Mapping

Association mapping, also known as linkage disequilibrium mapping was originally developed to study genetic disorders in humans (D'Hoop et al. 2008). Association mapping is a general approach to detect correlations between genotypic and phenotypic variation in a population based on the property of linkage disequilibrium. As compared to linkage mapping, which requires the use of highly related individuals such as full sibs, association mapping can exploit the properties of more complex populations with various degrees of relatedness. This approach can provide much higher levels of resolution for the genetic dissection of quantitative traits. Association mapping is considered as an alternative approach to the traditional QTL mapping. Association mapping has several advantages over traditional QTL mapping i.e. no need to develop segregating generations, a collection of various cultivars and breeding lines can be utilized for mapping studies and higher mapping population may be reached with many more meiotic recombination. In view of the advantages and applications of association mapping, it can be applied in potato to develop molecular markers for drought tolerance.

The feasibility of association mapping in tetraploid potato to identify QTLs for agronomically important traits like plant maturity and quality traits (D'Hoop et al. 2008, 2014), allele diversity (Kloosterman et al. 2013), and fry color (Byrne et al. 2020) using diverse potato genotypes. Berdugo-Cely et al. (2017) analyzed 809 andigenum group accessions from the Colombian Central collections (CCC) using SNP markers. They revealed that CCC is a highly diverse germplasm collection genetically and phenotypically and useful to implement association mapping in order to identify genes related to traits of interest and to assist future potato genetic breeding programs. Genome wide association studies (GWAS) are usually applied to large collections of theoretically unrelated individuals, the genetic diversity is supposed to be high and new alleles can be discovered. Furthermore, the high number of ancestral meiosis that occurred in the GWAS population can allow a precise QTL mapping. GWAS are much useful in diverse germplasm which offer new perspectives towards the discovery of new genes and alleles especially for complex traits like abiotic stresses in plants. Figure 2.2 summarizes the use of integrated molecular approaches for abiotic stress management in potato.

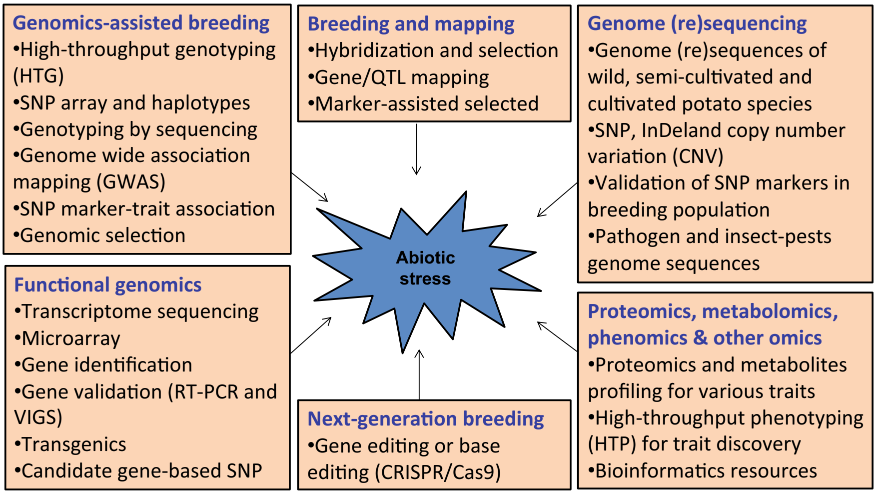

Fig. 2.2 Use of integrated molecular approaches for abiotic stress management in potato

2.7 Marker Assisted Selection

Since last two century tremendous progress has been made in molecular biology which provided important tools for plant breeders to accelerate genetic gain. Conventional screening in potato breeding program is performed using field trials, glasshouse trials and laboratory tests in order to identify genotypes with a combination of superior desirable traits. Nearly 40–50 characters are evaluated before development of the new variety. Most of these characters are quantitatively inherited and make the selection less efficient. Among the molecular tools, molecular genetic markers linked to the trait of interest can be utilized for faster development of new varieties. Marker assisted selection can be applied at the seedling stage and it eliminates classification errors due to environmental effects on phenotypic evaluation. It selects the traits early in the breeding program saving several years than the conventional breeding program. It is more effective for the qualitative traits controlled by major genes than the quantitative characters controlled by the minor genes. It can be effectively applied to quantitative characters if QTLs' of large effect contribute to the measured trait. A number of molecular markers have been reported in potato for biotic stress resistance but very negligible in abiotic stress tolerance, and of which only a handful has been employed in practical potato breeding (Barone 2004).

Potato map is one of the most highly saturated maps with different markers and also genome sequencing of potato has provided an ample opportunity for optimal use of DNA analysis for MAS. The most widely used classes of markers in current plant molecular breeding practice are SSRs and SNPs. These markers are generally highly polymorphic, codominant, reliable, relatively simple and cheap to use, and they can also be multiplexed (Collard and Mackill 2008). SNPs are abundant in potato. MAS helps in introgeression of genes from one genotype to another and most genome over 99% can be recovered with only three backcross generations instead six to seven in tradition backcross breeding program. While introgression from the wild species, many undesirable characters will be transferred which are reduced by positive and negative selections. Molecular markers closely linked to the gene controlling the trait of interest can be selected at the early stage without waiting for the specific development stage (positive selection). It reduces time and space and concurrent analysis of more than one trait is also possible. Negative selection can be applied to select the lines exhibiting less wild genome content and linkage drag around the introgressed gene. The availability of molecular markers specific to the donor genome will help to recover the highest recipient genome (Barone 2004).

The increasingly widespread availability of the molecular markers linked to single resistance genes and to QTLs for yield and qualitative traits may offer many new potential applications for the MAS in the production of new commercially available potato cultivars. Genetic marker systems and genome sequencing technology have become more sophisticated and cheaper to apply. Continued improvements in genomics technology and computational analysis, the costs of the MAS are expected to fall still further, permitting its application to a broadened range of traits in potatoes, as in other crops (Slater et al. 2013, 2014). Marker assisted selection for stress tolerance traits lags far behind MAS for disease resistance genes, because tolerance to theses stresses is often mediated by many interacting genes and factors (Watanabe et al. 2011). Potato responses to drought are mediated by a number of genes including transcription factors (Ambrosone et al. 2011). Anithakumari and coworkers (2011) identified drought resistant genes related to root-shoot ratios which are relatively controlled by few QTLs. These can be many potential candidates for development of linked molecular markers. Responses to drought are highly varied. With availability of complete potato genome sequence, potential development of markers will be facilitated (Ramakrishnan et al. 2015). Similarly many genes were identified for other abiotic stresses viz., cold, heat and salinity stress, but no markers have not been identified for MAS.

2.8 Genomics Assisted Breeding

2.8.1 Genome Sequence

The first reference genome was completed in 2011 using the whole genome shot gun approach by the Potato Genome Sequencing Consortium (Potato Genome Sequencing Consortium 2011). The source for the potato draft genome was DM1-3 516 R44 referred as DM and size of the potato genome is 840 Mb. Two years after the publication of the first reference genome, a new assembly of the DM clone was released with a more accurate arrangement of scaffolds and pseudomolecules (Sharma et al. 2013). This updated assembly of the potato reference genome (version 4.03) was created by integrating linkage data from a segregating diploid potato population derived from the reference sequence clone (DM). Recently an update of the potato reference genome (*S. tuberosum* group Phureja DM1-3 v6.01) has been released (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml).

S. commersonii is a wild species carrying important agronomic traits like resistance to root knot nematode, soft rot and blackleg, bacterial and Verticillium wilt, *Potato virus X*, common scab, late blight, and the ability to acclimate to the cold/freezing conditions. In 2015, the draft genome sequence of *S. commersonii* was released using whole genome shot gun approach with smaller genome size (830 Mb) than the potato genome. The smaller size may be due to the lower frequency of the transposable elements (TEs) found relative to the cultivated potato and tomato. The divergence time between cultivated potato and *S. commersonii* was estimated to be approximately 2.3 million years ago (Aversano et al. 2015) and 37,662 genes were predicted from the transcriptome analysis. The wild potato has fewer R genes than the cultivated potato and tomato. A total of 855 genes were differentially expressed in plants acclimated to frost stress and non-acclimated plants. Recently, Kyriakidou (2020) assembled genome of six polyploid potatoes including one Chilean and five Peruvian potato landraces which ranged from triploid to pentaploid (3x to 5x). These polyploid genomes provide a great resource to enhance potato breeding.

2.8.2 Gene Annotations

Structural annotation of the potato (DM) genome assembly was performed by the PGSC and the International Tomato Annotation Group (ITAG) (Potato Genome Sequencing Consortium 2011). The ITAG structural annotation of the potato genome was initially completed to allow for more direct comparisons with the ITAG structural annotation of the tomato genome, as both genomes were annotated using the same computational pipeline. Both structural annotations yielded approximately the same number of predicted genes. The PGSC and ITAG structural annotations have been included within a public online genome browser (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml). Functional annotation is a main area in which biological information is assigned to genes. Annotation of a large set of ESTs with a combination of similarity searches against protein databases and gene ontology (GO) terms were used to determine important genes under various stress conditions. Similarly, functional annotations have been determined for both potato and tomato defined gene models. A resource has been developed that integrates the PGSC and ITAG functional annotations together allowing researchers to search based on annotation and also visualize associated Pfam domains, and matches to UniRef, Arabidopsis, potato, and tomato proteins. The structural and functional annotation resources provide valuable information that is useful towards advancing potato and other Solanaceae, and increasing the efficiency of potato breeding.

2.8.3 Functional Genomics

The generation of EST collections is a primary source for large-scale gene discovery for many years in several crops including potato. These ESTs were an important source for genes and markers (SNP and SSR) and microarrays. However, now with the availability of whole genome sequence, ESTs are of lesser importance. ESTs generated by pooling across tissues and stress treatments show an importance transcriptome resources in terms of transcription factors, stress response genes, and signal transduction processes in response to abiotic stresses. These sequences in conjunction with RNA-seq reads were instrumental in the annotation of the potato genome assembly.

Functional genomics identifies genes and their associated functions and interactions with gene families at spatial and temporal levels. This requires experimental tools like high throughput gene expression analysis (transcriptomics), bioinformatics, contemporary analysis (metabolomics and proteomics), phenotyping and gene functions techniques. Transcriptomics aims to assess changes in the transcriptomes; proteomics studies the total protein content and metabolomics aims at study of small molecule resulting from the various metabolic processes. Several studies have been conducted for gene expression analysis, proteome analysis and metabolomics (Nakabayashi and Saito 2015). Cheng et al. (2019) analyzed WRKY members of different Solanaceous crops including tomato, potato and pepper using comparative genomics. Such finding helps to study the evolutionary relationships and development of stress tolerance varieties of potato and tomato.

The aim of the different omics approaches is to acquire comprehensive, integrated understanding of biological processes (Davies et al. 2008) to identify various players for drought and heat stress (Aksoy et al. 2015). The complex and varying nature of the environmental perturbations require a systems biology approach to delineate genes, proteins, and metabolites to properly assess the qualitative and quantitative changes and effects caused by the respective stress condition. However, despite current advancements in the "omics" technology, researchers lack good correlation between the data obtained across different platforms. Changes at the transcript level do not always reflect the alterations at the protein level, which may be majorly attributed to the degradation of transcripts and posttranscriptional regulatory mechanisms. Several studies have offered key insights into gene-level changes and regulatory interplay that occur during specific or combinatorial stress conditions (Bokszczanin et al. 2013). Transcriptome analysis shows numerous genes involved in various abiotic stress tolerance such as heat (Tang et al. 2020), drought (Moon et al. 2018; Chen et al. 2019), salinity (Li et al. 2020) and nitrogen deficiency (Tiwari et al. 2020a, 2020b, 2020c). In parallel, proteomics and metabolomics platforms have also been well-explored to elucidate the underlying complexity of various stress responses (Aghaei et al. 2008).

Proteomics studies are particularly informative which includes quantitative protein profile, protein–protein interaction network and their functions. There is an increasing use of proteomic approach to elucidate the complex relationship between stress tolerance and crop productivity. This information is especially important to identify specific potential biomarkers to come up with smart crop varieties known as proteomic-based marker assisted selection. Proteomics has now become an indispensable tool for high edge development in technology. With advancements in proteomics (mass spectrometry (MS)-based techniques) and quantification methods, proteomics has become a complementary tool for other omics and systems biology approaches (Weckwerth 2011, 2014). Comparative proteome analysis indicates the fine tuning metabolism as a major factor of stress tolerance of potato (Boguszewska-Mankowska et al. 2020).

Metabolomics focuses on the study of dynamics of low molecular compounds which results from the complex metabolic processes in the cell. The intensity of these processes is under the influence of both biotic and abiotic stress factors. Plants synthesize plenty of compounds of various chemical structures. These compounds are generally grouped as those of the primary metabolism which ensure the existence of any living being, and of the secondary metabolism which are characteristic of certain groups of organisms. Recently, gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography-mass-spectrometry (HPLC-MS), ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS), capillary electrophoresis-mass spectrometry (CE-MS) and nuclear magnetic resonance (NMR) spectroscopy are used to separate and identify metabolites (Hong et al. 2016). Advanced technologies and uniform extraction and detection methods, and available databases for the identification of various compounds, as well as multivariate statistics methods provide excellent opportunities for metabolomics studies.

2.8.4 Genome Wide Selection

Potato improvement applying next-generation breeding techniques is essential to shorten the breeding cycles. The availability of potato genome sequencing allows discovery of novel genes and markers associated with biotic stress tolerance and yield contributing traits. In order to achieve this, partitioning of genetic variance and genome wide prediction with allele doses is important in tetraploid potato (Endelman et al. 2018). In addition to conventional methods, new techniques have emerged for rapid crop improvement. GWAS and genomic selection (GS) based on high-throughput genotyping (HTG) by sequencing (GBS) and SNP array coupled with high-throughput phenotyping (HTP) and bioinformatics are powerful tools for rapid breeding of new varieties (Uitdewilligen et al. 2013; Vos et al. 2015; Sharma et al. 2018; Caruana et al. 2019). A few genome level studies in potato indicate starch phosphorylation associated SNPs by GWAS analysis using Illumina 22 K SNP potato array (GGP Potato V3) (Khlestkin et al. 2019), GBS for diversity and genomic selection in tetraploid potato (Caruana et al. 2019), HTP for rapid advancement of genetic gain in crop breeding programs (Zhao et al. 2019) and genetic diversity in CIP genebanks using SolCAP 12 K SNP array (Ellis et al. 2018).

Current cultivars continue to be susceptible to a range of biotic and abiotic stresses, and so are capable of significant improvement for a range of complex characters. Conventional potato breeding is relatively slow, laborious and a difficult process to obtain genetic gain. Modern breeding methods and technologies offer great benefit for the enhancement of breeding programs, but still require validation and cost-effectiveness in order to be adopted in practical breeding. In particular, MAS has the ability to select for traits earlier than conventional screening methods. MAS will be more effective for qualitative traits, but will also be valuable for quantitative characters, especially if QTLs of large effect contribute to the measured character, or if a group of markers can be identified that are linked to a group of alleles of smaller effects influencing the trait.

Animal breeding programs have adopted the estimation of breeding values using best linear unbiased prediction (BLUP), to exploit the additive genetic variance. Recently, the use of BLUP estimated breeding values (EBVs) in potato breeding clearly demonstrated the advantage of using EBVs over progeny means in cross-generation prediction of performance, particularly for traits with low heritability (Slater et al. 2014). As the BLUP analysis uses information from all relatives in the analysis, more data is used to calculate the EBV. EBVs represent the additive genetic effect transmitted from parents to progeny. This process enables identification of genotypes based on properties of the population in addition to individual phenotypes, and provides a more accurate selection process for such traits, and potentially enabling breeding from the best progeny much earlier (Slater et al. 2014).

MAS can reduce the duration of the breeding cycle between crossing programmes in order to combine these genes with other desirable traits. A combination of MAS with EBVs for complex traits will result in a significant reduction in the breeding cycle for all measured traits. Consequently, the use of both of these breeding tools will ensure rapid progress in combining traits and improving genetic gain in potato breeding programmes. Therefore, adoption of MAS and EBVs will provide substantial benefits within a multi-trait breeding strategy, and allow the design of superior combinations and their analysis for maximum genetic gain. Previous studies have shown that MAS can be cost effectively applied at the second field generation (Slater et al. 2013), and EBVs can be calculated for more complex traits at the same stage (Slater et al. 2014). These advances will greatly accelerate the breeding cycle, as the combined use of MAS and EBVs can reduce the breeding cycle from over 10 to as few as 4 years, and therefore, accelerate genetic gain relative to conventional breeding methods. At the same time, they will ensure that improvement is made in all measured traits, from those under simple genetic control to those under far more complex control.

2.9 Recent Concepts and Strategies Developed

2.9.1 Genome Editing

Highly heterozygous and tetrasomic inheritance nature of cultivated potato has made research complicated and time-consuming and therefore necessitates gene editing (Andersson et al. 2017). Given that efficient genetic transformation protocol and sequence information are available, potato is a good candidate for gene editing (Butler et al. 2015). There are many challenges that need to be addressed in establishing potato mutants using programmable nucleases. Genome editing which creates novel allelic variants in the genome uses sequence specific nucleases (SSNs) includes old techniques like ZFNs (Zinc Finger Nucleases), TALENs (Transcription Activator-Like Effector Nucleases), and recent CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR- associated proteins (Cas9) system. Among them, CRISPR-Cas9 system is an emerging next-generation breeding technology and an RNA-guided approach to target DNA sequence (now RNA also). Recent reports on the genome editing of major crops of economic importance including potato have shown high efficiency of SSN platforms for site directed precise mutagenesis (indels) of desired gene for resolute modification (Dangol et al. 2019). Owing to limitations with commercialization of genetically modified organism (GMO) in Europe or elsewhere, CRISPR-Cas is the most powerful genome editing tool. CRISPR/Cas9 is accounted for efficient site directed mutagenesis and gene silencing in potato (Tiwari et al. 2020d). In a study, CRISPR/Cas9 was designed with two sgRNAs to target *StALS1* gene (responsible for herbicide resistance) in *Solanum tuberosum* (Butler et al. 2015). Genome editing of the potato *GBSS* gene involved in amylase synthesis showed CRISPR/Cas9 system would be desirable for novel germplasm development, with targeted gene knockouts without any stable integration of DNA (Andersson et al. 2017). Genome editing in elite germplasm may be pursued for promoting alleles controlling desired traits and missing in the breeding populations.

2.9.2 Nanotechnology

Conventional breeding have met a limited success in improving stress tolerance due to complex traits, low genetic variability and inefficient selection methods. Despite so much progress, crop yield has reached a plateau in most crops including potato. This requires intervention of modern technology like nanotechnology to increase in food production from the available land area through efficient resource utilization while saving environments (Das and Das 2019). Nanotechnology is one of the most promising areas in the era of agricultural biotechnology (Usman et al. 2020). Nanotechnology explores wide area and opens large scope for diverse applications in fields of biotechnology, agricultural sector and health benefits (Cattaneo et al. 2010). Nanoparticles commonly referred to as nano scale particles (NSP's) size between 1 and 100 nm, and having extreme small size nanoparticles (NPs) have unique and diverse physical and chemical properties such as increased reactivity, expanded surface area, flexible pore size and diverse particle morphology (Sanzari et al. 2019). In the current scenario nanoparticles can be a potential to be affectively used as plant growth and development promoters, herbicides, nanopesitcides, nanofertlizers and so many others.

Abiotic stresses such as drought, heat, salinity, cold, nutrient deficits, chemical toxicity or oxidative stress are one of the major causes of crop loss worldwide. The application of nanoparticles or nanodevices causes both positive and negative impacts on various plant growth and development stages. Zinc oxide nanoparticles are involved in growth, flowering and seed productivity in onion (Laware and Raskar 2014). Nanotechnology promises the significant effort subjected to various traits in crops. In potato, Gowayed et al. (2017) suggest that the use of SiO_2-NPs at 50 mg L^{-1} as optimized dose improves plant growth under salinity stress through increased expression of genes and proteins associated with salt stress responses. Under stress conditions, ROS scavenging-related metabolic pathways are triggered, such as shikimate-phenylpropanoid biosynthesis and ascorbate and aldarate metabolism. Such over accumulated ROS in plants under abiotic stress can be scavenged by nanoparticles such as CeO_2, C60 and Fe_2O_3, NPs showed better performance under stress conditions, and further NPs enhance photosynthesis by light harvesting, electron transfer and ROS scavenging capacities (Zhou et al. 2021). Nanomaterials can alleviate the damage resulting from different abiotic stresses through activating process of plant defense system. Due to the properties of nanomaterials they can regulate water uptake by plant tissues, hence promote seed germination and plant growth (Zhou et al. 2021).

2.10 Genetic Engineering

*2.10.1 C*isgenesis

Climate change has a tremendous negative impact on agriculture production. Due to increase in population, demand for food is increasing rapidly. Hence there is a need to breed crop varieties with increased yield simultaneously able to cope up abiotic stresses besides diseases and pests which requires effective utilization of the genetic diversity. Traditional crop improvement methods involve genetic crossing, selection of the natural or artificial mutations, somatic hybridization and transgenics for the trait of interest. Genetic crossing from the wild species is time consuming due to crossing barriers, linkage drag and several generations are required to develop a line. Transgenic plants which involve introduction of gene from the different or same species or different kingdom brought considerable concerns about safety and impact on health and environment. Recent progress in pant genome sequencing has facilitated isolation of genes from crossable species and such genes are known as cisgenes. Such genes allow the plant to be modified while remaining plants within the same gene pool. Since, linkage drag is another problem faced by the breeders while transferring genes from wild species into cultivated type and it takes several generations to recover the recurrent parent genome. Using cisgenesis approach, late blight resistance genes were transferred to cultivated potato from wild potato species *S. bulbocastanum* (van der Vossen et al. 2003). It involves only the interested genes transfer along with native promoter, introns and terminators. Genotype and phenotype of the varieties remain unchanged and breeders have higher knowledge of transferred sequences. Cisgenesis can be applied in potato for abiotic stress tolerance as plenty of wild species are available containing number of genes for such traits. Thus cisgenic insertion of additional copies of native genes may provide a new approach to modify plant genomes, expand genetic variance in plant architecture available to breeders and accelerate the transfer of alleles between species which are difficult to cross.

2.10.2 Gene Stacking

The growing population and emerging environment challenges demand the development of more productive crops, more resistant to pests and diseases and tolerant to many stressful threats such as high salt, drought, flood, freezing and adaptation to poor quality agricultural land. In addition, the need to save water in agriculture is compelling the development of crops more efficient in its utilization. Gene stacking refers to introduction of two or more transgenes of agronomic interest in the same plants. Advances in genetic transformation technologies and genome sequencing have facilitated the introduction of multiple genes and characteristics in a single variety using gene stacking strategies. The multi-engineering in plants can make those goals more feasible to achieve. Examples of gene stacked crops are rice, wheat, canola, cotton, potato, soybean etc. In potato GAANTRY (Gene Assembly in *Agrobacterium* by Nucleic acid Transfer using Recombinase technologY) system was used to develop transgenic plants with stacked transgenes (McCue et al. 2019). Plants with stacked genes now form a significant part of GM crops grown throughout world just like gene pyramiding in breeding. In the future, tolerance to abiotic stresses would be priority in changing climate scenario.

2.11 Bioinformatics

Bioinformatics is an indispensable tool to analyze genome level data generated through high-throughput next-generation sequencing technologies. With the increasing genomics resources and reducing cost, it opens more avenues for the use of bioinformatics in plant research. Genomics and post-genomics research necessarily requires the use of modern bioinformatics tools for genomics, transcriptomics, metabolomics, proteomics and phenomics data analysis and also for repository of enormous amount of data. Enormous amount of nucleotides (DNA and RNA) and protein sequence data are deposited with the international gene banks. The most commonly used resources for data submission are NCBI (National Centre of Biological Information) in USA (http://www.ncbi.nlm.nih.gov), EMBL (European Molecular Biology Laboratory) in Europe (http://www.ebi.ac.uk/embl/), and DDBJ (DNA Data Bank) in Japan (http://www.ddbj.nig.ac.jp). Besides, there are a number of various other bioinformatics tools used for data analysis.

The potato genome sequence, deciphered in 2011 by the Potato Genome Sequence Consortium, is maintained by the SpudDB, Potato Genomics Resources, Michigan State University, USA (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml). This website describes about the potato genome sequence, annotation, search tools, genes and various other information for breeders and biotechnologist. Recently, this database has been updated with recent version of the potato genome sequence and the genome sequence of M6, a wild potato species (*S. chacoense*). Moreover, a dedicated genomics resource is available for Solanaceous crops i.e. Solanaceae Genomics Network (http://solgenomics.net/). This includes a collection of genetic maps, genomes, and tools. PoMaMo (Potato Maps and More) () provides information about maps, anchor markers, genes and variants. The PlantGDB (http://www.plantgdb.org/StGDB/) database has data on genome, gene model, alignment, gene structure annotation and annotated protein alignment. Importantly, there are several international potato gene banks for germplasm exchange such as the International Potato Centre (CIP) (https://cipotato.org/), Lima, Peru; the US Potato Genebank (NRS) USA; the CGN Potato Collection at the Centre for Genetic Resources, the Netherlands (CGN); and the Commonwealth Potato Collection (CPC) of the Vavilov Institute (VIR). CIP is amongst the largest international potato gene bank in the world, which provides potato germplasm throughout the world. A number of country-specific databases have been developed such as the European Cultivated

Potato Database (ECPD) (https://www.europotato.org/), UK potato variety database (http://varieties.ahdb.org.uk/). Thus, there are several potato-based databases in many countries for research and development.

2.12 Social, Political and Regulatory Issues

The potato industry involves protection of intellectual property rights (IPRs) related to industrial applications, such as processing, product development, and trademarks. Biotechnological inventions are widely covered by patents. Seed-tuber production and quality assurance have a strong association with proprietary technology, such as that on tissue culture, diagnostics, biotechnological products like genes and processes, and technical aspects of breeding may be protected under IPRs. New varieties can be protected by plant variety protection laws under the International Union for the Protection of New Varieties of Plants (UPOV). In the public sector, including state universities, has filed and owns a variety of patents rather than use the UPOV scheme. A patent on genetic markers can be made to allow a diversity of free public users of markers. Genes have been protected under the IPR system, and applications for commercial use often have been hindered due to the difficulty of integrating patent licenses. Financial resources and/or cross-licensing deals need to be considered for IPR coordination. Private–public cooperation could provide an open arena for the utility of IPRs. This will provide flexibility to the potato breeders by allowing free IPR platforms for cultivar development.

CIP has pioneered in the area of participatory research (PR) which includes farmer back to farmer, integrated management of pest and diseases, participatory variety selection, farmer field school, participatory approaches for native potato variety value chains using the participatory market chain analysis, advocacy for PR and policy change, ending with nutrition-related PR in more recent years. The farmer-back-to-farmer model emerged from an interdisciplinary CIP team that included both social and biophysical researchers. Researchers must work with farmers to identify the right problem to solve, interact creatively with them, work on-farm and on-station, and present the results back to farmers for feedback. Further, an agronomist at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) developed a new method called the mother-and-baby trial design (MBT) for participatory research with farmers. The MBT method, also known as the central/satellite design, involves a complete, replicated trial in a central location in a community, with satellite mini-trials in farmers' fields. The method was widely used at CIMMYT and elsewhere as well. At CIP, Lima the mother-and-baby trial design allowed plant breeders to understand farmers' selection criteria (e.g. large tubers, resistance to frost and to late blight). Farmers in the highland communities participated in assessing CIP's improved clones from a breeding population. This helped not just to select desired traits, but to facilitate registration of new varieties and to disseminate them with farmers, buyers, processors, and consumers.

Adaptation and resilience to climate change requires farmers to adapt faster information. ICTs offer the possibility of connecting a larger number of farmers with sources of information and advice to make decisions to tackle climate change. The essence of PR approaches has been maintained over the years as a mechanism to facilitate the dialogue between scientists and farmers. PR method needs to include a larger number of viewpoints from the innovation and agri-food systems. New ICTs offer the possibility of improving communication, analysis, and decision making systems. World over social scientists, breeders, pathologists, and agronomists have been continuously working together to create influential participatory methods with farmers and different stakeholders with the help of emerging technologies.

2.13 Future Perspectives

Crop designing based on genomics resources is need of the day for potato improvement. The information presented about various abiotic stresses affecting potato growth and development such as genetic resources, genetic diversity, classical genetics and breeding, gene mapping, marker assisted selection, genomics assisted breeding, recent concepts (genome editing and nanotechnology), genetic engineering, bioinformatics tools and social political and regulatory issues. In climate change scenario, vertical expansion through increasing productivity is one of the possible approaches via intervention of genomics resources. This will also help in fast breeding of potato varieties. Introgression of yield enhancing genes/QTLs and early stage selection through genomic selection coupled with stress tolerance would be feasible to enhance productivity. Identification of new genes and markers in wild resources would be required for diverse genetic background for tolerance to various abiotic stresses such as heat, drought, salinity, nutrients and frost. The introgression of genes from wild resources will be important to widen the genetic diversity of the cultivated potato through modern genomics approaches like gene editing or base editing. Given that wild relatives are important source of various tolerance genes to abiotic stresses and therefore integration of genes and use of molecular markers especially genomics-assisted approaches is necessary for expansion of productivity. Furthermore, horizontal expansion of potato cultivation under nontraditional areas particularly tropical regions where high temperature and other environmental factors prevail is necessary to break the yield barriers. The expansion in nontraditional areas would require integrated approach for various abiotic factors along with early stage selection through genomics selection and yield enhancement. Taken together, there is immense potential for horizontal and vertical expansion of potato cultivation through both cultivation in nontraditional areas and productivity enhancement. This is possible through introgression of genes from wild/semi-cultivated/cultivated into the cultivated potato gene pool, reducing breeding cycles via genomic selection, abiotic stress tolerance genes, gene pyramiding, next-generation breeding and yield enhancement.

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Chapter 3
Molecular Approaches for Breeding Abiotic Stress Tolerance Traits in Capsicum Species

John Momo, Khushbu Islam, Nitin Kumar, and Nirala Ramchiary

Abstract *Capsicum* is an important member of the Solanaceae family grown worldwide for its fruits which are utilized as spice as well as a vegetable crop. *Capsicum* fruits are highly rich in pharmacological compounds such as carotenoids (provitamin A), vitamin C and E, flavonoids and the distinct metabolite alkaloid complex known as capsaicinoids which impart pungency to its fruits. Like other crop plants, *Capsicum* suffers major yield losses and quality setbacks due to adverse abiotic conditions such as drought, salinity, extremely high and low temperatures and humidity. These environmental constraints limit the productivity and affect the physiological growth of *Capsicum* by altering osmotic balance, affecting growth, reproduction, fruit ripening and quality. Therefore, *Capsicum* needs urgent attention from the plant researchers and breeders in order to reduce losses due to environmental conditions. In this chapter, we review the current state of abiotic stress tolerance in *Capsicum* spp. expanding over the natural germplasm, molecular breeding and resistance mechanisms. Marker-assisted selection and genome-wide association studies for the useful exploitation of resistance genes and QTLs have offered considerable advantages over the conventional plant breeding approaches for the improvement of *Capsicum* in terms of accuracy, specificity and duration. Furthermore, the next-generation sequencing technologies have proven breakthroughs in the field of identification of the genomic regions responsible for stress tolerance, evasion and responses which could be employed for future *Capsicum* breeding programs.

Keywords Capsicum · Drought · Salinity · Heat · Humidity · Resistance genes

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3.1 Introduction

3.1.1 Economic Importance of **Capsicum**

The origin of *Capsicum* (chilli pepper) cultivation is dated to approximately 9000 years ago in Central and South America. It is believed to have been distributed by "New world" traders to Europe and various parts of the world (Kraft et al. 2014). Domestic cultivation began around 2000 years back due to the increasing economic and pharmaceutical value of this plant. Fruits of chilli plants have become increasingly demanding for its characteristic culinary purpose in tropical and subtropical regions of Africa, India, Far East and Latin America (Olatunji and Afolayan 2018). From its early ancestral form, chilli plants have been domesticated and bred for its highly economic value and its pharmacological properties that includes the presence of carotenoids (provitamin A), highly rich vitamin C and E, flavonoids and its distinct metabolite capsaicinoids that play role in scavenging free radicals, as pain reliever and also in radiation therapies and cancer (Simone et al. 1998). Now they are grown in million hectares of land annually all around the world. The cultivation and production of chilli plants has grown dramatically over the years reaching almost 3.8 million ha of area under cultivation and 38–40 million tons of green and dry chilli peppers produced during the year 2019 (FAO; www.fao.org). The compound Capsaicin has been increasingly important in pharmaceutical and medicinal uses and evolutionarily interesting in deciphering the evolution of secondary metabolic pathways. Pungent, non-pungent and moderately pungent varieties of *Capsicum* are now cultivated. *Capsicum annuum* is majorly cultivated as a food crop and *Capsicum chinense* and other pungent varieties are cultivated for their spicy trait. Important carotenoid compounds in chilli plants include capsanthin, capsorubin, beta carotene, beta cryptoxanthin, lutein, violaxanthin, zeaxanthin (Mohd Hassan et al. 2019). The capsaicinoids in pungent chillies are believed to have been evolved to deter mammalian predators from consumption. The compound is sensed by neurogenic peptides and TRPV channels to cause a burning sensation (Naves et al. 2019). In contrast, capsaicinoids have pharmacological significance viz. anti-inflammation, carminative, diaphoretic, emetic, spasmolytic and tonic properties (Popelka et al. 2017).

3.1.2 Reduction in Yield and Quality Due to Abiotic Stresses

Capsicum suffers from adverse environmental conditions and stresses. Abiotic stress viz. heat, salinity, drought, cold etc. limit the productivity of *Capsicum*. The biochemical composition of bell pepper fruits were found to be affected by the water stress conditions, resulting in delayed ripening and altered osmotic balance (Borràs et al. 2021). These abiotic stresses often occur simultaneously or sequentially and prove detrimental to the overall growth and reproduction and thereby endangering food security. The signalling response to abiotic stress conditions in plants is complex and encompasses the molecular, cellular and physiological mechanisms in the plant tissues (Zhu 2016). Climate change and the deteriorating environment have exacerbated the problem by posing fresh threats to the agricultural sector and produce and therefore there is an immediate need for the development of crop varieties with tolerance to multiple stresses. The increasing demand for better crops have rendered *Capsicum* challenging in production, as its growth is highly affected by various biotic (fungi, virus, nematodes, aphids, bacteria) and abiotic factors (heat, salt, drought, cold, metals). Recent reports suggested accumulation of heavy metals particularly Cadmium (Cd) and Lead (Pb) in *C. annuum* plants following the treatment with organic fertilizers which may pose a health risk (Ugulu et al. 2021). These factors in conjunction with abiotic stress conditions like salinity, drought and adverse temperatures viz. heat and cold affect the soil and limit the productivity and quality of the *Capsicum* crop.

3.1.3 Genetic Advancements and Challenges in **Capsicum** *Crop Improvement*

The conventional plant breeding approaches offer a number of limitations in terms of accuracy, specificity and duration as opposed to marker-assisted selection (MAS) and breeding for desired traits. Identification of genome-wide SNPs and their association studies offer greater advantages over linkage mapping for the useful exploitation of QTLs and genes that confer stress resistance in crop plants. Whole genomic-selection by assessing breeding value based on total molecular markers and their phenotypic manifestation is a more successful and preferred method of crop improvement in plants. Further, next-generation sequencing technologies have proven breakthroughs in identifying and examining the genomic regions responsible for stress tolerance, evasion and responses. Genomic-assisted breeding has revolutionized the area of plant breeding with the state-of-the-art techniques that have contributed more than we can ever imagine achieving in the years to come for coping with ever increasing population and food demands.

Breeding in *Capsicum* has been greatly improved from the conventional methods by the development of novel markers, QTLs, mapping designs and techniques in association with latest NGS technologies. Many QTLs, markers and genes have been identified in *Capsicum* for physiological, molecular and biochemical assessment of resistance against abiotic stress, disease resistance, and pungency traits. Some *Capsicum* germplasms have been reported in India for tolerant varieties (Reddy et al. 2014). They include fungal resistant varieties viz. Bhut Jolokia PBC80, LLS, Breck-1, Breck-2 and Jaun that are resistant against *Colletotrichum* spp., BS35, GKC29 against blight disease caused by *Phytophthora capsici* and bacterial resistant varieties viz. GKC29, PI201234, IC364063 that are resistant against bacterial wilt strain 'MC-4'. Genetic advancements have resulted from efficient mapping of the genome of

Capsicum. Attempts at initial mapping were done by contemporary techniques about a decade back by polymorphic RFLP markers (Lefebvre et al. 1993), AFLP (amplified fragment length polymorphism), SSRs (simple sequence repeats), EST-SSRs (expressed sequence tag derived simple sequence repeats), SNPs (single nucleotide polymorphisms) and CAPs (Cleavage amplified polymorphic sequence) (Cheng et al. 2016). Now, with advanced technologies and developments in sequencing *Capsicum* genome, pools of datasets are available (Kim et al. 2014b; Qin et al. 2014; Ahn et al. 2018).

Compared to other model plants such as tomato or tobacco of the same family Solanaceae, protocols are yet to be standardized for transformation and efficient transgene integration in *Capsicum* spp. Much of the study is limited to characterizing the functions of genes by mutation studies (Choi et al. 2014), gene identifications (Reddy et al. 2014) and metabolite study (Wahyuni et al. 2011). Therefore, a thorough understanding of in-depth mechanisms is a potential arena for exploration in *Capsicum.*

The elucidation of stress resistance mechanisms and trajectories in *Capsicum* demands robust transcriptomic sequencing directed towards the spatial and temporal pinpoints of environmental signalling pathways. The availability of high-quality and well annotated genomes complemented with massive amounts of transcriptome data relating to the various climatic conditions and environmental signals can provide assistance in the selection of desirable agronomic traits to aid in breeding programs. For the development of stress tolerant *Capsicum* species, the basic understanding of signalling and response at molecular, cellular and physiological levels is crucial. Crop improvement programs for developing stress-resistant cultivars in the long run have employed traditional breeding methods to modern day state of the art techniques such as next generation sequencing (NGS). Transcriptome sequencing has been widely accepted as an easy and reliable method for studying the plant responses and mechanisms to abiotic stresses and identifying the target genes involved in the defense mechanisms in the plant tissues. Our current knowledge of abiotic stress-related mechanisms and plant responses is limited at the molecular level and an urgent call of action is needed to handle eminent realities.

3.2 Abiotic Stresses in *Capsicum*

3.2.1 Heat, High Humidity and other Stress

Capsicum grows well in a moderate environmental temperature ranging from 21 to 33 °C (Thuy and Kenji 2015). Varying high and low temperatures from the optimum affect the physiological growth viz. germination, fruit settings, fruit maturity and rate of metabolite accumulation especially the synthesis of capsaicinoids as well as the reproductive stage and overall productivity (Guo et al. 2016). While several physiological disorders associated with heat stress have been reported in *Capsicum*, there are still a lot of studies to be conducted at the molecular level to understand the mechanisms that drive the plant's survival against high temperatures**.** Higher temperatures for a longer duration of time affect the germination rate, developmental and physiological parameters in *Capsicum* spp. (Rosmaina et al. 2021). At the molecular level, an important factor that plays a universal role against high temperature in plants is the heat shock proteins (Hsps). Under high temperature, a cascade of signalling events generate secondary messengers like Ca^{2+} which further activate a network of proteins further downstream. Whenever a stress triggers a hypersensitive response in plants, heat shock transcription factors bind to heat shock elements at the promoter region to regulate the expression of genes that alleviate heat stress. Hsps are itself a diverse family of proteins that regulate the physiological and biochemical response of plants to heat stress and other stress as well. They range in varying molecular weights from 20 upto 100 kDa and are named accordingly as Hsp100, Hsp60, Hsp90, Hsp70, Hsp20 (Sarkar et al. 2019). They play a ubiquitous role in protein folding and assembly and also aid in preventing protein disaggregation during denaturation. cDNA cloning of a full length 779 bp cytosolic class I HSP (accession AY284925) was done whose expression was induced by heat treatment (Guo et al. 2005).

A comprehensive study has been conducted on CaHsp20 and CaHsp70 families in *Capsicum annuum* L. (Guo et al. 2016) where several organelle localized transcripts were found under thermal treatment that were directed to the cytoplasm, mitochondria, golgi bodies and the chloroplast and ectopic expression of cytosolic genes that regulated proteins conferring resistance to heat were determined (Table 3.1). Each of the family of chaperonins plays more or less similar roles by a network of conjunctions between them with several signaling factors playing a role in between. They also share homologous functions with other plants belonging to Solanaceae viz. tomato (Yu et al. 2016) and potato (Li et al. 2010). Hsp60 are reported to play a critical role in the growth and development of *Capsicum* as well as in several stress responses (Haq et al. 2019). Several variants of the Hsp family that possess the functional amino acid motifs of the protein family are also known to be showing regulation and enhanced activity during high temperature like CaHsp22.0 (Sun et al. 2019) and CaHsp25.9 that confer multi-tolerance to heat, salt and drought (Feng et al. 2019), both belonging to the CaHsp20 family. These proteins also interact with each other and also with other family members of transcription factors, regulators and enhancers (Feng et al. 2019).

WRKYs were earlier known to directly regulate the expression of Heat shock proteins (Hsps), ethylene and ABA responsive genes and also other WRKYs under high temperature and humidity stress and in response to *Ralstonia solanacearum* infection in *Capsicum* (Cai et al. 2015b). *CaWRKY40* was later reported to specifically up-regulate the transcription of a *C. annuum* mildew resistance locus O (*CaMLO6*) in the root and leaves under high temperature and high humidity stress (HTHH). *CaWRKY40* downregulated under *Ralstonia solanacearum* infection in the roots and VIGS induced *CaMlO6* silencing significantly reduced HTHH resistance which confirms the specificity of transcription factors like WRKY in temperature

Table 3.1 Heat shock proteins (Hsps) and other factors in combating heat stress in *Capsicum*

Family	Functions/role	References
CaHsp20	Thermotolerance (irreversible aggregation of proteins)	Guo et al. (2015a), Sun et al. (2019)
CaHsp25.9	Induced thermotolerance by increase in accumulation of MDA, SOD, proline, and photosynthetic activity	Feng et al. (2019)
CaHsp16.4	Increased thermotolerance by increased MDA, CAT, SOD enzymes and higher chlorophyll content	Huang et al. (2019)
CaHsp70	Thermotolerance	Guo et al. (2016)
CaHsp60	Protein folding and refolding	Haq et al. (2019)
CaHsp60.6	Knockdown confer enhanced sensitivity to heat stress	Haq et al. (2019)
CaHsp40 Or DnaJ family	Molecular chaperones involved in growth and development and alleviation of heat stress	Fan et al. (2017)
Autophagy related Ubiquitin family (ATG)	Autophagy and regulation of protein degradation	Zhai et al. (2017)
CaChiVI2 (CBP)	Thermotolerance and resistance against *Phytophthora capsici*	Ali et al. (2020)
CaBiP	Protein folding and aggregation in Endoplasmic reticulum	Wang et al. (2017)
CaWRKY27	Negative regulation of thermotolerance	Dang et al. (2018)
CaWRKY6	Transcriptional activator of *CaWRKY40* confers resistance to High humidity and high temperature	Cai et al. (2015b)
CaWRKY40	Directly influence gene expression of *PR* (pathogenesis related) and Hsp in response to high temperature	Dang et al. (2013)
CaWRKY40b	Modulates the expression of *CaWRKY40* and enhances the expression of *CaWRKY40, JAR*, *RLK1*, *EIN3*, *FLS2*, *CNGIC8*, *CDPK13* and heat shock cognate proteins	Ifnan Khan et al. (2018)
CaDIR7 (dirigent gene family)	Lignification and protection from high UV rays	Khan et al. (2018)
NAC family	Interaction with MAPK to alleviate heat stress	Diao et al. (2018), Zhang et al. (2020a)
Wall associated kinase gene *CaWAKL20*	Negatively regulates thermotolerance by reducing the expression of ABA responsive genes	Wang et al. (2019)

(continued)

Table 3.1 (continued)

Family	Functions/role	References
CaHsfA1d	Thermotolerance by increased expression of Hsp and antioxidant gene *GSTU5* (glutathione-S-tranferase Tau 5)	Gai et al. (2020)
CaHSL1 (*C. annuum* HAESA-LIKE receptor like protein kinase)	Regulated by *CaWRKY40* and induce thermotolerance by increased transcription of *CaHsp24* and improved photochemical efficiency of PSII both in light and dark	Guan et al. (2018)
CaHsfB2a	Binds to *CaWRKY6* and *CaWRKY40* to regulate high temperature and high humidity stress (HTHH) by curtailing the expression of *CaHIR1*, *CaNPR1*, *CaABR1* and *CaHsp24*	Ashraf et al. (2018)
CaHsfA2	Alleviate heat stress at multiple regions, roots, leaves, stem, pericarp and placenta	Guo et al. (2015b)
CaZNF830	Increased transcription under heat and humidity stress by regulating the expression of *CaHIR1, CaNPR1, CaABR1, CaPR1* and *CaHsp24*	Noman et al. (2018)
CDPKs and CRKs (CDPK related kinases)	Increased transcription in response to heat and other stress	Cai et al. (2015a)
CaDofs	Differentially expressed in response to heat and salinity stress	Wu et al. (2016)
GSTSs (Glutathione-S-transferase family)	Heat stress, growth and development	Islam et al. (2019)

stress (Yang et al. 2020). Similar activity was observed for a *C. annuum HAESA-LIKE (HSL)* receptor like protein kinase (RLK), *CaHSL1* that was upregulated by HTHH but not by *Ralstonia solanacearum* infection (Liu et al. 2018b) and for a *C. annuum* zinc finger transcription factor (CaZFN) in which case transcription was elevated by both HTHH and *R. solanacearum* infection (Noman et al. 2018). High temperature causes formation of abnormal petals, stamens and gynoecium in flowers of *C. annuum* L. (Polowick and Sawhney 1985). In tomato, high temperature resulted in a higher ratio of maleness which was because of a *stamenless 2* gene and cold temperatures promoted the female characteristics and flower formation (Heslop-Harrison 1972). High GA3 in capsicum plants shows abnormalities in petal and stamen formation and reduces pollen viability (Sawhney and Shukla 1994). Plant hormone ethylene causes rapid leaf abscission that is accelerated by water, drought and osmotic stress (Gomez-Cadenas et al. 1996). $AgNO_3$ and $CoCl_2$ influence flowering of *C. frutescens* in vitro by inhibiting ethylene synthesis and action (Sharma et al. 2008). Humidity also decreases floral opening and anthesis by affecting the vegetative growth (Barker 1989).

3.2.2 Cold Stress

Plants undergo chilling injury when the ambient temperature is below 10–15° Celsius (C) above freezing point. There are observations of membrane composition changes, leakage of electrolytes, increased malondialdehyde, Reactive Oxygen Species (ROS) and decreased proline content and finally plasmolysis. Plants adopt a general mechanism of survival strategy against effects from chilling and freezing injury viz. synthesis of antifreeze proteins, accumulation of compatible solutes, proline, amino acids and sugar alcohols etc. Chilli plants are warm-season perennial plants that don't do well in cold temperatures. Temperatures below 7–10 °C have been shown to retard growth. Some of the symptoms of chilling injury in chilli plants include seed and calyx browning, surface pitting, tissue discoloration, restricted shelf life and quality, watery strains, superficial pitting, damage due to dehydration and increase in hydrolase in the membrane of cells (Wang and Balint-Kurti 2016). Chilli plants under 8 °C for 24 h start showing flaccidity of stem and leaves accompanied by nitrosative and oxidative stress which is shown by increase in protein tyrosine nitration and lipid peroxidation (Airaki et al. 2012). The symptoms of cold stress like seed browning can be inhibited upon Methyl Jasmonic Acid (MeJA) or induced Methyl Salicylic Acid (MeSA) treatment (Seo et al. 2020). Early studies of cold stress in *Capsicum* deduced the relative concentration of respiration rate shown by reduced accumulation of ubiquinone (Q) and alternative oxidase (AOX) which resulted in increased oxidative damage due to increased respiration and reduced oxygen (Popov et al. 2001).

The manifestations of reduced tolerance to low temperature may severely affect the physiology and morphology of the plants. Few studies have been performed for elucidating the genes and mechanisms of low temperature tolerance in plants, even lesser in *Capsicum.* A local chilli cultivar of Seychelles, *sy2*, is a temperature-sensitive natural mutant of *C. chinense* which shows developmental defects at temperatures below 24 °C. The phenotypic characterization and genetic mapping of this mutant has revealed the temperature-dependent response mechanisms in *Capsicum.* The *sy2* mutants grown at 20 °C showed deformed leaves, fewer chloroplasts, impaired chloroplast development, broader epidermal cells and collapsed mesophyll cells in the leaf tissues (An et al. 2011). High amounts of ROS-H_2O_2 and superoxide were generated in the chloroplasts of *sy2* mutant leaves under 20 °C temperatures. Low temperature treatment also reduced the expression of fatty acid biosynthetic pathway genes like *Cafad7* (linolenic acid) in *sy2* mutants compared to the wild type and *sy2* mutants at 26 °C.

Another *C. chinense* cultivar, Tr-13, a local landrace from Trinidad and Tobago (Carribean country) did not develop true leaves or developed morphologically abnormal leaves at temperatures below 24 °C (Koeda et al. 2013). Wild type (*C. chinense* No. 3341) from Bolivia was taken as a control. Temperatures lower than the threshold induced the expression of defense-related genes in Tr-13 in the absence of pathogen infection similar to *sy2* mutant and in contrast to the wild type (*C. chinense*

No. 3341) (Koeda et al. 2013). Elevated levels of Salicylic acid (SA), an important hormone in plant defense mechanisms, were observed in the leaves of Tr-13 at 24 °C. Interestingly, both *sy2* and Tr-13, when grown in temperature conditions simulating their place of origin, showed latescent developmental abnormalities. Several defense-related genes were expressed in Tr-13 grown at simulated temperature but the endogenous levels of SA were similar to those at constant temperature of 25 °C. The *sy2* plants grown in the simulated environment neither expressed defense-related genes nor observed increased SA accumulation. Molecular phylogenetic studies have indicated that temperature-sensitive Tr-13 and *sy2* are distantly related (Koeda et al. 2013).

3.2.2.1 Regulatory Genes and Molecular Mechanisms

The plasma membrane is the first barrier affected under cold or other abiotic and even biotic stress. Therefore, membrane stability is important in rendering resistance to incoming threats. The plasmalemma and plastid membrane damage was analyzed by microstructural and low field NMR in *C. annuum* to reveal the role of membrane components monogalactosyldiacylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid as key biomarkers under cold treatment at 4 °C. *Capsicum* plants responded by significantly increasing its electrolyte content, proline accumulation and phospholipase activity (Kong et al. 2018). In the later year, the transcriptome profile of *C. annuum* was reported in response to cold treatment which led to the identification of 3363 Differentially Expressed Genes (DEGs), with 1669 upregulated and 2194 downregulated genes (Kong et al. 2019). The upregulation of a CaF-box predominantly in stem and seeds reported in *C. annuum* under cold stress revealed by lipid peroxidation and cellular death under CaF silenced lines (Chen et al. 2014). Recently, *CaPLD alpha4* (phospholipase D) gene was found to be associated with the stability of plasma membrane and its activity increased at 4 °C. Its expression was associated with upregulation of *CaNAC1* suggesting their interaction and regulation and was proved by yeast one-hybrid experiment (Kong et al. 2020).

Hsps are important for protein and biomolecular stability. *CaHSP26* plays an important role in protection of photosystem (PS) II by regulation of antioxidative enzymes and cis-unsaturated fatty acids to avoid photooxidation and increasing fluidity of the plasma membrance under chilling stress (Li et al. 2012). In colder non-tropical areas, integrated stress induced by pathogens and cold temperature is common. Early screening for molecular regulators identified two wound inducible MAP kinases MK1 and MK2 that were constitutively expressed when attacked with biotic pathogens and also elevated in cold stress (Shin et al. 2001). *CaPF1*, an ERF/AP2-type transcription factor was revealed to regulate the expressions of genes that contain a GCC or a CRT/DRE box under an integrated stress effect of cold temperature and *Xanthomonas* and *Pseudomonas* infection (Yi et al. 2004).

Many genes regulate multiple biotic and abiotic stress and they crosstalk by signalling to regulate the expression under the specific stimuli (Table 3.2). Among

Table 3.2 List of genes reported to be induced during drought stress in *Capsicum* species

Gene/gene family	Area of maximum transcript accumulation under drought	Influence by other biotic and abiotic factors	References
Class II chitinase gene (*CaChi2*)	Stem and leaf	*C. coccodes*, ABA	Hong and Hwang (2002)
Stellacyanin (*CaSLP1*)	Phloem and xylem	*X. campestris*, *C. coccodes*, *P. capsici*, *C. gloeosporioides*	Kong et al. (2002)
CASAR82A	Phloem and epidermal cells	*X. campestris*, salinity and low temperature	Lee and Hwang (2003)
Zinc finger protein gene (*CAZFP1*)	Leaf, root and stem	*X. campestris* cv. Vesicatoria, ABA	Kim et al. (2004)
Drought inducible *DS2* genes (conserved in potato, tomato and capsicum)	Leaves under dehydration stress	ABA, salinity, cold and hypoxia or oxidative stress	Dóczi et al. (2005)
Pepper pathogen induced *CaPIP2*	Localized in cytoplasm mostly in leaves	Salt stress and *Xanthomonas campestris* pv. vesicatoria	Lee et al. (2006)
Pepper C3-H-C4 type RING finger protein gene *CaRFPI*	Leaves	*Colletotrichum coccodes*	Hong et al. (2007)
Pepper antimicrobial protein gene *CaAMPI*	Leaves	Salinity and ABA	Lee and Hwang (2009)
Pepper xyloglucan hydrolase *CaXTH3*	Leaves and roots	Salinity	Choi et al. (2011)
Pepper RING E3 ubiquitin ligase *CaRma1H1*	Endoplasmic reticulum localized in leaves	Salinity	Seo et al. (2012)
Pepper methionine sulfoxide reductases (MSRs)	Chloroplast in leaves and stem	Reactive oxygen species	Kim et al. (2014a)
Acidic SK3 dehydrins	Phloem cells	Cold temperature	Szabala et al. (2014)
Pepper lipoxygenase *CaLOX1*	Constitutive localization	Osmotic and high salinity	Lim et al. (2015)
Pepper ethylene responsive transcription factor *CaAIEF1*	Leaves	Enhanced ABA sensitive	Hong et al. (2017)
Pepper bZIP and *CaDILZ1*	Leaves	ABA sensitive	Lim et al. (2018)
Chitin binding protein family *CaChilV1*	Constitutive	*Phytophthora capsici*	Ali et al. (2019)

(continued)

Table 3.2 (continued)

Gene/gene family	Area of maximum transcript accumulation under drought	Influence by other biotic and abiotic factors	References
CaHSP16.4	Constitutive expression	Heat stress	Huang et al. (2019)
Pepper MAP kinase *CaAIMK1* and *MEKK*	Nucleus and cytoplasm mostly in leaves	ABA and ROS	Jeong et al. (2020) Lim et al. (2020a)
Pepper Dehydrin *CaDHN3*	Nucleus and cell membrane	Salt and ROS	Meng et al. (2021)

these, 41 transcription factor families were identified that were induced under cold stress among which the *DREB/CBF, NAC* (14%), *C2H2* (12%), *AP2/ERF* (12%), *JAR1/JAZ* and *MADS* families were most distinctive. More specifically, *CaNAC064* and *CaMADS* was researched upon (Chen et al. 2019; Hou et al. 2020) to deduce its role in cold tolerance where their downregulation resulted in wilting, higher chilling injury index, lower proline increase in ROS, increased electrolyte leakage and lower chlorophyll contents.

First report of expression profile of genes of *C. annuum* under cold stress identified transcription factors that were also simultaneously upregulated by salinity stress. They include the family of ethylene responsive elements viz. EREBP-C1-C4, bZIP protein (*CaBZ1*), ring domain protein RVA, HSF1 and CaWRKY1 (Hwang et al. 2005). A cold responsive C-repeat/drought responsive element binding factor (CBF/DREB 1b) family, *CaCBF1A* and *CaCBF1B* were specifically expressed at critical 14–15 °C and reported to interact with homeodomain leucine zipper protein (HD-Zip) (Kim et al. 2004). Even a heterologous expression of *Capsicum CBF3* gene was conducted to confer cold tolerance in transgenic tobacco (Yang et al. 2012). Aquaporins are a part of major intrinsic proteins (MIPs), thus far identified in higher organisms, that transport water and neutral molecules across the cell membrane and maintain the water potential (Tyreman et al. 2002). *Capsicum* aquaporin gene *CaAQP* helped in decreasing the effects of chilling stress by triggering the stomatal opening in transgenic tobacco plants and its loss of function causes severe growth retardation (Yin et al. 2014). An attempt was made to identify genes and ESTs by suppression subtractive hybridization (SSH), where 37 unigenes were predicted by gene ontology analysis and expression of upto 10 ABA responsive genes were confirmed for their expression under cold stress (Guo et al. 2013).

Proteomic reports of *Capsicum* under chilling injury brought to the understanding of physiological and metabolic changes in the plant. *Capsicum* proteomes under cold stress show increased ethylene production, malondialdehyde (MDA), and relative alterations in redox homeostasis, carbohydrate metabolism, ascorbate glutathione cycle, glucose metabolism, Calvin and Krebs cycle (Sánchez-Bel et al. 2012). Two bell pepper cultivars, commercial hybrid (Canon) and abiotic stress tolerant S103 accumulated leaf metabolites particularly caffeate and benzoate and root metabolites gamma aminobutyric acid (GABA), proline, galactinol and raffinose under chilling injury at 7 °C suggesting a more effective regulation of photosynthesis induced by stress related metabolism (Aidoo et al. 2017).

Acidic dehydrins SK(n) is constitutively expressed under cold stress particularly in the sieve elements and companion cells of roots and stems in Solanaceous crops and homologs in *Capsicum* and tomato were suggested to render protection to the phloem vessels (Szabala et al. 2014). *CaDHN1* was cloned in the later year in *C. annuum* and its expression was positively correlated with cold induction (Chen et al. 2015). A genome wide identification was carried out to screen for *CaDHN* genes that were upregulated in response to cold and osmotic stress (Jing et al. 2016). *C. annuum* putative U-BOX protein 1 CaPUB1, an E3 Ubiquitin ligase is conserved in monocots and dicots and overexpression of *CaPUB1* in rice positively regulated and conferred tolerance to rice (Min et al. 2016).

GRAS family of transcription factors in *Capsicum* also regulate abiotic stress and was predicted to show signalling crosstalk with Giberellic Acid (GA) responsive cis-elements (Liu et al. 2018a). Candidate auxin transporters *CaLAX/CaAUX* and *CaPIN* regulate various abiotic stresses including cold temperature for polar transport and root elongation (Zhang et al. 2018).

Transcriptome study of bell pepper in response to cold treatment suggested the role of long non-coding RNAs (lncRNAs), miRNAs (microRNAs) and circular RNAs (circRNAs) which were putative targets of various classes of transcription factors viz. *Myeloblastosis (MYB)*, *basic helix-loop-helix* (*bHLH)* and *Ethylene Response Factors (ERFs)* (Zuo et al. 2018). A *NAC (NAM/ATAF1/2, CUC2*) transcription factor regulates cold stress in *C. annuum* and is induced by exogenous application of Abscisic Acid (ABA) and SA (Zhang et al. 2020a). *CaMADS* transcription factors are known to regulate multiple abiotic stresses including cold. MADS expression was downregulated in Virus induced gene silencing (VIGS) induced silenced lines showing impaired growth in plants and increased electrolyte leakage, MDA content and reduced chlorophyll (Chen et al. 2019).

3.2.2.2 Negative Regulators

A *Solanum tuberosum* homolog of *Capsicum CaMBF1* which encodes a coactivator multiprotein bridging factor 1 negatively regulates cold tolerance and plants displayed electrolyte leakage and cell lysis manifested by visible symptoms of leaf damage (Guo et al. 2014). *Capsicum* is rich in carotenoids and the level of carotenoids is negatively regulated by the *CaATHB-12*, a key gene belonging to the *HD-ZIP I* family of transcription factors (Zhang et al. 2020b). *CaATHB-12* overexpression lines decreased the antioxidant enzymes and silenced lines positively upregulated the antioxidant enzymes.

3.2.2.3 Cold Management Strategies

Seed priming of *Capsicum* using thiourea imparts cold tolerance to the developing plants by further increased expression of key regulators of chilling injury viz. *CaWRKY30, PROX1, Cu/Zn Superoxide Dismutase (SOD)* and some osmoprotective enzymes (Patade et al. 2012). The seed germination of *Capsicums* can be increased upto 8% in cold temperature by exogenous treatment with chitosan and additives like trisodium phosphate (TSP) and SA to improve enzymatic glucanase or chitinase activity and to enhance water retention efficiency (Samarah et al. 2020).

Glycine betaine have been shown to enhance cold tolerance by maintaining a higher ratio of unsaturated/saturated fatty acids in tomato (Anunziata et al. 2019), *Arabidopsis* and tobacco (Shi et al. 2018) along with proline to provide osmotic and chilling protection. Exogenous applications of ABA have shown to increase tolerance to chilling injury in *Capsicum* by enhancing the expression of oxidative scavenging enzymes viz. *SOD, Catalase (CAT)* and guaiacol peroxidase activity (Guo et al. 2012). Combination of trisodium phosphate (TSP) and SA treatment in *Capsicum* under cold temperature (4 °C) increased fatty acid desaturation shown by upregulation of fatty acid desaturase gene and higher unsaturated fatty acids (Ge et al. 2020). Exogenous application of glutathione rendered chilling alleviation by lowering lipid peroxidation, peroxide and superoxide reduction by upregulating genes of the Ascorbate–glutathione cycle (AsA-GSH) viz. *CaAPX1, CaGR2, CaMDHAR1* and *CaDHAR2* (Yao et al. 2021). Treatment of MeJA and 1-methylcyclopropene (ethylene inhibitor) in cold treated *Capsicum* upregulated several genes (upto 470 genes upregulated and 291 genes downregulated) for peroxidase, CAT, ERF, Mitogen-activated protein kinase (MAPK) and JA biosynthesis (Shin et al. 2017). Hot water treatment to chilling injured chilli plants were reported to show chilli recovery from damage. This was supported by metabolite analysis of hot water treated cold stressed plants where several sugars, polyamines and phenolics shot up (López-Velázquez et al. 2020). Grafting *C. annuum* cultivars ‘Chifengtexuan’ as scion and ‘Weishi’ as rootstock obtained decreased cold related parameter viz. lower electrolytes and MDA content with increased *SOD, Peroxidase (POD)* and *Ascorbate Peroxidase (APX)* (Wang et al. 2010).

3.2.3 Drought Stress

Capsicum is a popular plant in the Mediterranean region where it is mostly affected by drought. They are sometimes known to be commonly susceptible to drought and water deficit areas due to their large transpiration and leaf surface areas and high stomatal conductance. Drought results in cell flaccidity, dehydration, membrane damage, tissue discoloration, loss of plant integrity, accumulation of ROS and Reactive Nitrogen Species (RNS) and reduced photosynthesis caused by reduced water potential (Bojórquez-quintal et al. 2014). It physiologically affects the seed germination, seedling emergence, fruit settings and flower formations and induces early senescence (Demir and Mavi 2008). Tolerance to drought is focussed on factors that restore the osmotic balance in the tissues viz. compatible solutes like proline, sugars, amino acids, glycine betaine and ROS scavenging enzymes.

A number of *Capsicum* accessions were assessed for tolerant cultivars viz. Numex X (A31), Numex X (A31), Numexsandia type 2 (A32), Numex conquistador type 2 (A33), BGV-11814 (A34), BGV-4349 (A35), SIURIYA 600 (A36) and KAPIYA UV (A37) to study their response to drought induced PEG treatment (López-serrano et al. 2017). A31 and A34 were shown to have low effect upon drought induction. Grafting of tolerant variety (A25) with non-tolerant have also been observed to absorb water better, have better water retention capacity including sustained CO_2 assimilation rate in roots and increased nitrate reductase activity in leaves to survive in water deficiency (López-serrano et al. 2019). A novel DREB1 type *Ca-DREBLP1* in *Capsicum* that resembles in form with that of *A. thaliana* has been reported to be induced upon drought, osmotic stress, salinity and mechanical shearing (Jong-Ping Hong and Woo Taek Kim 2005). *CaBZ1*, a bZIP transcription factor family, was upregulated in response to osmotic and drought stress (Moon et al. 2015). *Capsicum* showed tolerance to drought and oxidative conditions when a *sod* gene encoded *Cu/Zn SOD* from tomato was trangenetically introduced which also improved shoot regeneration (Chatzidimitriadou et al. 2009). This gives us insights into check-mating the drought solution in *Capsicum* plants for long term production and agricultural benefits. A molecular marker from *Capsicum SAR8.2* gene designated as *CASAR82A*, B and C isolated from hypersensitive leaves infected with *X. campestris* was found to regulate multiple stresses including drought. *CASAR82A* expression was induced upon drought in the phloem and epidermal tissues of *Capsicum* plants (Lee and Hwang 2003).

One of the earliest studies of *Capsicum* in drought conditions identified a novel mutant causing flaccidity of cell (*flc*) generated by treating seeds using ethyl methane sulfonate (EMS) which later indicated that the mutant was due to a recessive gene by inheritance studies (Bosland 2002). During water deficiency, the stem of *Capsicum* plants induces the early expression of *Cachi2* genes whose roles correspond to the floral and endodermic expression when infected with fungus such as *Colletotrichum coccodes* (Hong and Hwang 2002). Some of the genes or gene families whose expressions are induced by drought stress are given in Table 3.2.

The capsaicinoid biosynthetic genes *Phenylalanine ammonia lyase (PAL), Cinnamate 4-hydroxylase (C4H), POD* and *Capsaicin Synthase (CS)* showed altered levels of expression under drought in comparison to control and *PAL* in association with *CS* showed corresponding increase or decrease in response to drought (Phimchan et al. 2014). The capsaicinoids content in *C. annuum* were found to be increased during drought conditions as compared to the excess water conditions (Mudiyanselage et al. 2020).

3.2.4 Salinity Stress

Saline soils affect the productivity of *Capsicum* at various stages starting from germination, flowering, fruitification, nutrient concentration etc. (Kpinkoun et al. 2019). It is a recurring threat to agricultural productivity worldwide estimating loss of upto 20% cultivated and 30% of irrigated lands (Shrivastava and Kumar 2015). *Capsicum* have been shown to be moderately sensitive, sensitive and highly sensitive to saline soils (Aktas et al. 2005). Soil upto 4 dS/m (approximately 40 mM NaCl) affects the growth of plants causing high osmotic imbalance, ion toxicity, nutritional disorder and soil infertility. Salts are present in the form of ions (Na^+, Cl^-, K^+, Mg^+) in soil and are manifested through weathering of soil, improper irrigation and leaching from soil profile by precipitation (Aslam et al. 2011). Na^+ is the most abundant ionic salt that causes soil salinity followed by Cl, Mg, K, B and Ca. Ion toxicity in plants is because of K^+ ions replaced by Na^+ and Cl^- ions. These ions cause changes in protein conformations and disrupt the normal physiological and biochemical metabolism (Zhu). In India, upto 7 million hectares of land mostly from the Gangetic region involving states like Haryana, Uttar Pradesh, Punjab and Rajasthan are saline (Srivastava et al. 2019).

Like any other plant, *Capsicum* too follows a Na^+ extrusion or Na^+ sequestration into vacuole under saline stress (Bojórquez-quintal et al. 2014). Mitigation is accompanied by proline accumulation in resistant varieties and ionic compartmentation carried out by K^+ transporters, Na^+/K^+ antiporters Salt Overly Sensitive (SOS1) and intracellular NHX antiporters (Fig. 3.1). *C. annuum* cv. Taranto was investigated for its response to varying irrigation water salinity and deficient irrigation at different developmental stages which revealed the higher level of irrigation water alleviated the salinity effects and the vegetative stage is more tolerant than reproductive stage. Effects of salinity were studied on callus regeneration and growth in *C. annuum* that concluded the accumulation of capsaicin, ascorbic acid, phenylalanine and proline helps in tolerance to salt stress and that the stress treated callus tissues can tolerate the salt stress in field conditions (Al Hattab et al. 2015).

The roots of *Capsicum* show positive response and growth in saline soils. *Capsicum* undergoes modification of distribution pattern by shifting photosynthetic assimilates from shoot to root (Polash et al. 2019). This results in an increasing surface area of roots and physiological reduction in K^+ and $P0^{4+}$ salts. Recently, a Manganese Nano Priming was reported to improve root growth and prime compositional change in molecular interactions to mitigate salt stress (Yuqing Ye et al. 2020).

3.2.4.1 Biochemical, Molecular and Biological Control Mechanisms

There are many factors that play a role in alleviating salt stress in plants and particularly in *Capsicum*. While the total fruit set is affected by saline soils, the content of capsaicinoid is shown to be increased upon salt stress (Kpinkoun et al. 2019).

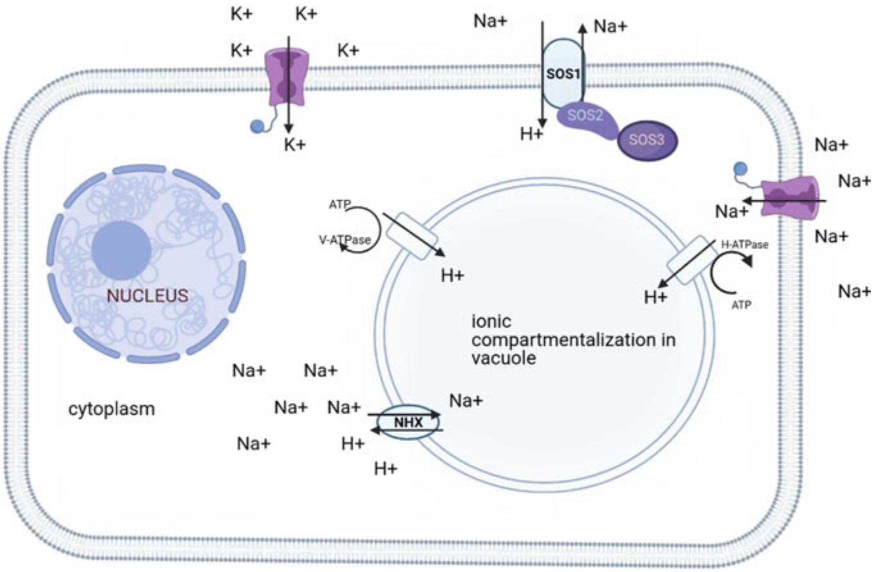

Fig. 3.1 General representation of Salt Overly Sensitive (SOS1) pathway in plants for sequestration of toxic ions mediated by vacuole

Hypersalinity tends to cause reduction in the essential mineral elements in the plants like N, K, P, Si, Zn and chlorophyll content all of which play important biochemical and physiological roles in metabolism. *Capsicum* also synthesizes compatible solutes like any other plants to survive the osmotic dehydration and flaccidity of internal tissues viz. proline, glycine betaine, polyamines etc. At the molecular level, Dehydrin responsive genes *CaDHN5* in *Capsicum* has been shown to improve salt and osmotic tolerance (Luo et al. 2019) and transcription factors *CaMADS* (Rugong Chen et al. 2018) and *CabZIP25* (Lin et al. 2020) positively modulates salt tolerance.

An exogenous application of silicon and glycine betaine is shown to improve the plants uptake of essential mineral elements like N, K, P, Zn and chlorophyll content and this conferred tolerance to salinity (Hussein and Abdullah 2019). Also, application of cinnamic acid and polyamines putrescine, spermine and spermidine mitigate salt stress damage in *C. annuum* by elevating endogenous NO levels, flavonoids, proline and K^+/Na^+ ratio (Lin et al. 2020) and upregulating radical scavengers like *SOD*, *CAT* and tissue electrical conductivity (Seedling et al. 2019). Hormonal application to induce intracellular scavenging mechanisms is possible like NO induced by exogenous Salicylic application to upregulate Ascorbate Glutathione cycle to combat hyperosmotic cellular damage caused by salinity (Kaya et al. 2020).

Micro-organisms also play a role in mitigating salt tolerance. *Pseudomonas putida* RS-198, a gram negative soil bacterium was shown to improve the *SOD* (25.22%) and polyphenol oxidase (78.06%) activity of *C. annuum* upon inoculation in saline soils. Some bacteria that survive in saline habitats when treated to chilli improved the N, P, K and proline levels (NururAimi et al. 2019) and Arbuscular mycorrhiza *Glomus* *constrictum* mitigated salt tolerance in *Capsicum* (Al Amri et al. 2019). Microorganisms have also been effectively used with biochar in saline soils to reduce Na^+ and Cd^+ salts and increase dehydration tolerance, nutritional status and irrigation use efficiency of *Capsicum* (El-mageed et al. 2020).

3.3 Physiological Disorders

3.3.1 Blossom-End Rot of Capsicum

Blossom-end rot initially called "black rot" is a physiological disorder in plants like tomato, chilli, eggplant, water-melon and cucumber that causes sunken, water soaked, dark brown rot in the distal parts of the fleshy fruits. It is estimated to have caused crop losses upto 50% worldwide (Taylor and Locascio 2007). It was initially addressed by Raleigh and Chucka 1944 to be a result of calcium deficiency or inadequate Ca^{2+} concentration. The decrease in concentration of Ca^{2+} in the plants less than 0.20% over stimulated ethylene and low transport of Ca^{2+} particularly to the distal ends of fruits caused end rot (Marcelis and Ho 1999). The lack of Ca^{2+} in the cell wall and membrane compromise their integrity by losing permeability and causing leakage of cellular contents.

Max C. Suare in 2014 re-appraised that Ca^{2+} is not a critical factor. In fact, blossom rot was reported to be a result of multiple abiotic stresses (Taylor and Locascio 2007) viz., high salinity, high Mg^+, high NH^{4+}/K^+, inadequate xylem development, fluctuating high and low temperatures, accelerated growth rate, pH and use of high concentrations of fertilizers (Westerhout 1962; Wiersum 1966). He reported that Gibberellic Acid (GA), which decreases Ca^{2+} accumulation when itself down regulated, inhibited the formation of blossom rot even at optimum Ca^{2+} levels, thereby stating that Ca^{2+} was not a necessary critical element for the disorder.

Symptoms of blossom rot usually start appearing during the early spring due to increasing temperature and light intensity. Studies by Morley et al. (1993) and Marcelis et al. (1998) in *C. annuum* observed that the rapid growth is associated with induced blossom rot as in the case of tomato (Ehret and Ho 1986). Comparison of normal and blossom rot affected *C. annuum* in the open field have detected high levels of N, P, K and Mg in the distal part of the fruits isolated in the affected fruits (Lee et al. 2012). Root architecture also affected the uptake and Ca^{2+} distribution in the xylem sap, changing the dynamics of blossom rot in *C. annuum* (Karni et al. 2000). High salt in soil contributes to low root conductance and affected ion distribution to the aerial shoots. During a study by Hakan Aktas et al. 2004 treating *C. annuum* with salt, Ca^{2+} levels in fruits were not affected. Rather, a change in manganese and zinc levels were obtained in the leaves and fruits and change in Ca^{2+} was observed in the leaves. Blossom rot worsened in salt treated plants. Apoplastic ROS increased in the salinity treated plants by enhancing NAD(P)H oxidase activity resulting in decreased ions like manganese, zinc and calcium. Manganese and zinc were observed to scavenge apoplastic ROS and reduced blossom rot. A study conducted by Turhan et al. 2006 proposed a relationship between apoplastic ROS and Ascorbate activity with salt treatment in relation to blossom end rot. Ascorbic acid was reported as a protective mechanism against blossom end rot that resulted in pH imbalances and ascorbate oxidase enzymatic activity. For improvement against blossom rot, biostimulants have shown to decrease the rate of rot occurrence in *C. annuum* L. (Nada Paradikovic et al. 2013).

3.3.2 Sunscald

Sunscald occurs in fruits of plants exposed to high sunlight/heat. It results in white or yellow blisters with a papery flattened appearance. This leaves the affected areas to be vulnerable to bacterial, fungal, maggots and viral infections. It affects agronomically important crop plants viz. *Capsicum* (Weber 1932; Bremer 1945), tomatoes (Cook 1921; Ramsay and Link 1932), plums (Harvey 1925; Maxie and Claypod 1956), apples (Brooks and Fischer 1926), cucumbers (Ramsay and Link 1932), and oranges (Ketchie and Ballard 1968). The early breaker green parts of the fruits are usually more susceptible as in tomato and *Capsicum* compared to the red ripe fruits. This is probably seen as a result of the thermal stability of carotenoids supported by increased osmotic pressure in ripe fruits (Retig and Kedar 1967). In *Capsicum*, the damage caused by sunscald is shown to be variable in the detached and attached fruits, with detached pepper more vulnerable to UV exposure (Rabinowitch et al. 1983) evident from rapid chlorophyll disintegration leading to a highly susceptible photo-oxidative process (Fig. 3.2).

Tolerance to sunscald could be achieved for those natural plants that were pre-conditioned with heat through di-urnal changes in temperature (Rabinowitch et al. 1986). Overexpression of APX or SOD enhanced lipid peroxidation to protect from UV-B or grafting rootstocks that increase vitamin C content, photo-protective or PSII enhanced antioxidant cultivars could be employed (Wang et al. 2010). Mitigation of excessive mineral nutrients like Ca^{2+} or nitrogen that accelerates the symptoms of sunscald and proper irrigation management along with mechanical protection and shielding from extreme sun rays or UV sources can be practiced.

3.4 Identification of Genes Responsible for Tolerance Traits

Differential DNA hybridization and sequence analysis led to the identification and isolation of an ABA-induced *CaDIN1* gene from ABA-treated leaves of *C. annuum* cv. Hanbyul (Lim et al. 2014). The quantitative estimation using real-time PCR showed significant upregulation of *CaDIN1* on treatment with ABA, high salinity and drought in *Capsicum* leaves. The *CaDIN1* protein was found to be localized in the chloroplast using green fluorescent protein (GFP) tagging on the C-terminal region

Fig. 3.2 Sun scalding observed in *Capsicum* fruits

of *CaDIN1* containing construct (35S:*CaDIN1*: *GFP*). In a silencing experiment using the tobacco rattle virus (TRV) induced gene silencing and phenotype analysis, *CaDIN1*-silenced plants showed increased tolerance to drought stress under re-watering conditions. The *CaDIN1*-silenced leaves showed lower transpiration rates, lesser MDA concentration and higher *CaOSM1* gene expression than the control leaves. The *CaDIN1*-overexpressing lines (*CaDIN1-OX*) in Arabidopsis showed decreased sensitivity to ABA in seedling, stomata and roots and decreased tolerance to drought treatment. The expression analysis suggested that *CaDIN1* negatively regulates ABA and drought responsive genes—*RAB18*, *RD20a*, *RD22*, *RD26*, *RD29B* and *DREB2A* and in response to drought stress.

The '*sy2*' *Capsicum* mutant has two low-temperature-sensitive F-box proteins—*LTSF1* and *LTSF2* with important roles in low-temperature sensitivity. Both the proteins share 24–25% sequence identity with F-box-associated (FBA) motif containing Arabidopsis Constitutive Expressor of PR Genes 1 (CPR1) protein. The *LTSF1/2*-doubly silenced pepper (No. 3341) plants showed severely retarded growth and abnormally shaped thick leaves under low-temperature conditions (20 °C), similar to the '*sy2'* mutant phenotype (Venkatesh et al. 2020). Both *LTSF1* and *LTSF2* interact with S-phase kinase-associated protein 1 (SKP1) of the Skp, Cullin, F-box-containing (SCF) complex suggesting their roles in protein degradation. The *LTSF-1* overexpressing *Nicotiana benthamiana* plants grew taller than the control plants indicating tolerance to low temperature in transgenic lines. Also, *LTSF-1* transgenic lines showed increased accumulation of ROS-scavenging antioxidant enzymes in low-temperature stress conditions. The significant increase in gene transcripts of *CAT*, *APX*, *GST* (glutathione S-transferase) and *SOD* in transgenic lines during low-temperature conditions may influence antioxidant pathways. Two genes in the *sy2* locus were differentially expressed in the wild type No. 3341 and *sy2* mutant plants when grown at 20 and 28 °C temperatures. Overall three genes—*ORF26, ORF9* and *ORF10* were downregulated at low temperature in both wild type and mutant but the expression level of *ORF10* was 8.2 times higher in wild type at both the temperatures. Four genes (*ORF14*, *ORF8*, *ORF17* and *ORF20*) showed up regulation at low temperature in both wild type and *sy2* mutants. However, *ORF20* was 1.2 times more highly expressed at 20 °C and 1.8 times more highly expressed at 28 °C in the wild type than the *sy2* mutants. Therefore, *ORF10* and *ORF20* were differentially expressed under cold stress.

CaOSM1 (Osmotin-like protein) is observed to be induced upon abiotic stress like high salinity and cold-treatment and along with plant hormones like ethylene and MeJA (Hong et al. 2004). The *CaAIMK1* (ABA-induced MAP kinase 1) is known to play positive roles during ABA and drought stress (Jeong et al. 2020). MAPK signalling transduction pathways are integrated in plant growth and development and abiotic stress response. The chromosome 2 and 7 in *C. annuum* has nine closely located *CaMEKK* genes. Nine *MAPK* genes were shown to be transcriptionally regulated by ABA and drought stress in qRT-PCR expression analysis in *C. annuum* (Lim et al. 2020b). *MLO* locus associated with rapid cell death, validated by several overexpression studies, and has been a ready target to understand abiotic resistance. Silenced copies of it confer general resistance to drought stress (Lim and Lee 2014).

3.5 Molecular Mapping of Resistance Genes and QTLs

3.5.1 Mapping of QTLs

The *sy2* mutation was found to be a single gene recessive mutation (Koeda et al. 2012). The genetic analysis of *sy2* locus in the low temperature sensitive mutant *sy2* has been done using the F_2 mapping population obtained from cross between wild type (*C. chinense* No. 3341) and *sy2* plants grown at 20 °C (An et al. 2011). The *sy2* phenotype was due to a single recessive gene based on the typical Mendelian segregation ratio of 3:1 obtained in the F_2 population. In further studies, the *sy2* gene was precisely mapped to a 138.8 kb region in the distal portion of the chromosome 1 (Liu et al. 2016). The F_1 and F_2 population obtained from a cross between wild type No. 3341 and *sy2* were grown at low temperature (20 °C) and used to develop a high density genetic map. The conserved synteny analysis between pepper and tomato genetic map revealed a 126 kb gene coding region of the Tomato scaffold sequence (C01HBa0051C14) (http://solgenomics.net) corresponding to the *sy2* locus based on the two flanking COSII markers (C2_At1g09070 and C2_At4g29120) using

FGENESH (http://linux1.softberry.Com). Tomato scaffold sequence were used in BLAST search against *C. annuum* (CM334 V1.5) genome database (http://passport.pepper.snu.ac.kr/?t=CAB) and three homologous scaffold sequences in pepper were obtained—2607 (377.7 kb), 3515 (200.8 kb) and (2510 (318.6 kb). In the flanking region of COS21, genes coding for acyl-CoA6, heat shock proteins, lipoxygenases etc. were predicted. Further insight in this linkage group revealed the potential identity and nature of the *sy2* genes.

The genetic cross between *C. chinense* accessions Tr-13 and wild type No. 3341 revealed that the Tr-13 temperature-sensitive phenotype was caused by a single gene recessive mutation in the same gene locus as *sy2* (Koeda et al. 2013). It is important to conclude here that plants with temperature-sensitive phenotypes due to natural mutations avoid manual selection and thrive easily in specific environments.

3.5.2 Marker Development

Among the 91 COSII markers used for mapping 12 pepper linkage groups in the F_2 mapping population, six COSII markers including COS21 (C2_At2g15890) on the pepperlinkage group 1 were found to be linked to *sy2* phenotype (An et al. 2011; Wu et al. 2006). The flanking COSII markers—C2_At4g29120 (COS511) and C2_At1g09070 (C)S634, were located at a distance of 1.6 and 3.1 cM from the *sy2* locus, respectively. The comparative analysis of this region with the Tomato-EXPEN 2000 genetic map (http://solgenomics.net) located these two markers at 3.7 and 4.0 cM on the tomato chromosome 1.

The nucleotide sequences of the wild type *C. chinense* (No. 3341) and *C. chinense* mutant *sy2* were aligned and SNPs were detected in the flanking region of the *sy2* locus (Liu et al. 2016). Based on the pepper scaffold homologous sequences, SNP markers from the intergenic regions were amplified and used to map *sy2* genes. Out of the 16 identified markers for the *sy2* locus, eight High resolution melting (HRM) based SNP markers and four KASP (Kompetitive allele specific PCR) markers were used to generate a high-resolution genetic map for the *sy2* target region. Seven genes in the *sy2* locus showed sequence variations when the coding sequence was compared between wild type No. 3341 and *sy2* mutant. Out of the two SNPs identified, SNP A952G led to a change in amino acid (I318T) in the gene *ORF9*. Two SNPs identified each in *ORF20* and *ORF10* caused amino acid changes. In *ORF14*, three out of five SNPs identified caused amino acid changes. The sequence gap between two non-overlapping pepper scaffold sequences (3515 and 2510) were closed using 21 positive *C. annuum* (CM334) BAC (Bacterial Artificial Chromosomes) clones containing scaffold sequences.

The SNP and Kompetitive-allele specific PCR (KASP) markers developed were used to perform genotyping analysis for the fine mapping of the *sy2* locus (Liu et al. 2016). The *sy2* locus was found to be a 0.14 cM (138.8 kb) region enclosed between SNP5-5 and SNP3-8 markers on the pepper scaffolds 3515 and 2510. Four SNP markers—SNP5-3, SNP3-13, SNP3-12 and SNP5-1 were located at a genetic distance of 0 cM from the *sy2* locus. The *sy2* locus was flanked by eight KASP markers on one end and two SNP markers on the other end. Four SNP markers were located within the *sy2* locus. Within the *sy2* locus, about 27 ORF genes with diverse cellular functions were identified using BLAST searches in Tomato CDS (ITAG release 2.40) and Pepper CDS CM334 (V1.55) database. Out of these, four putative genes (one cullin-like isoform X1 (*ORF6*) and three F-box family proteins (*ORF20*, *ORF10* and *ORF24*)) were found to be coding for proteins of the SCF complex, a multiprotein E3 ubiquitin ligase complex. *A* typical F-box domain of approximately 50 amino acids is found in *ORF10* and *ORF20* at the N-terminal while it is absent in *ORF24* and therefore is a truncated F-box protein. ORF10 and ORF20 have functional domains like kelch repeats at the C-terminus.

3.6 Genomics-Aided Breeding for Resistance Traits

3.6.1 Details of Genome Sequencing

The whole genome sequence and assembly of hot pepper (*C. annuum*) cv. 'CM-334' was reported by (Kim et al. 2014b). The genetic variation and divergence between CM-334 and three other *Capsicum* species—*Capsicum* cultivars i.e. Perennial and Dempsey and a wild type *C. chinense* PI159236, revealed 0.35% (10.9 million SNPs) in Perennial, 0.39% (11.9 million SNPs) in Dempsey and 1.85% (56.6 million SNPs) divergence in *C. chinense* PI159236. Draft genomes for *C. annuum* genomes—a progenitor variety of Zunla1, Chiltepin (Qin et al. 2014) and a F1 hybrid resulting from cross between CM334 and a non-pungent pepper breeding line (Hulse-Kemp et al. 2018) were sequenced by whole genome shotgun approach via next-generation sequencing. The size of sequenced genomes was approximately 3.5 Gb and the number of annotated protein-coding genes were around 35,000. Also 6527 lncRNAs (5976 inter-genic and 222 are intron-overlapping), 5581 phased siRNAs and 176 miRNAs (35 pepper specific) were also reported by Qin et al. (2014). Several potential orthologous genes related to fruit development, disease resistance, genome expansion and evolution in *Capsicum* genome were identified through comparative genome analysis using Tomato Genome Consortium 2012 and Potato Genome Sequencing Consortium 2011.

Two high quality de novo genomes for *C. baccatum* PBC81 and *C. chinense* PI159236 and an refined reference genome for *C. annuum* CM334 were published using Illumina Hiseq 2500 with multiple-size insert genomic DNA libraries (Kim et al. 2017a, b). The estimated sizes for the *C. baccatum* and *C. chinense* genomes were 3.9 Gb and 3.2 Gb, respectively, and about 35,000 protein-coding genes were annotated in both the species using Pepper Genome Annotation (PGA), which were similar to 34,771 genes of tomato (Tomato Genome Consortium 2012).

3.6.2 Functional Genomics Resources

Presently, transcriptomic studies for diverse plant species treated under various forms of abiotic stresses are being done on a wide scale to gain insights into the plant defense responses. Few studies in the Solananeae family have articulated important aspects of the response mechanism during the abiotic stress responses. Potato tubers were imposed with severe drought conditions and re-watering treatment and transcriptomic sequencing was followed (Gong et al. 2015). The two groups of experimental stresses, along with the comparison group between the two, revealed the specific DEGs. Interestingly as many as 263 DEGs, many among them homologous to auxin-responsive protein, abscisic acid 8'-hydroxylases, calcium-transporting ATPase etc., showed contrasting expression patterns in the two stress conditions and turned out drought and water stimulus responsive. Among others that showed differential expression upon re-watering, 16 DEGs were related to starch synthesis and accumulation and tuber formation. Gene families like *MYB*, *bHLH*, *ERF*, *WRKY*, *NAC*, *bZIP* and *HD-ZIP* constituted for about 50% DEGs in the comparative analysis between the stress groups.

A study shows how the plants have adapted to simultaneous multiple stresses and, therefore, are potential resistance sources for breeding and development of multi-stress resistance crop plants (Rasmussen et al. 2013). The *Capsicum* transcriptome assembly has facilitated the generation of gene-based molecular markers for the selection of desired traits eventually useful in molecular breeding programs. The functional annotation of assembled genes is done based on the sequence homology to already annotated genes in several publicly accessible databases and can be assigned with GO (gene ontology) terms. The first high throughput transcriptome assemblies of *C. annuum* varieties—Mandarin and Blackcluster were sequenced using pyrosequencing. The detailed sequence variation analysis revealed 1025 and 1059 SNPs specific to the two genotypes, respectively (Ahn et al. 2013). The same next generation sequence assemblies were utilised for the discovery of 2067 and 2494 SSRs in the transcript regions of the two pepper genotypes (Ahn et al. 2014). The *C. annuum* cv. Bukang was used to generate a total of 187.8 Gb transcriptome data using 78 strand-specific RNA libraries. After adapter filtering and quality check using Cutadapt (Martin) and FastQC, the reads were mapped to *C. annuum* 'CM334' reference genome v.1.6 (https://peppergenome.snu.ac.kr) using Hisat2 (Kim et al. 2014b). The raw read counts were estimated using HTseq-count (Anders et al. 2014) and normalised to FPKM to quantify the gene expression values. Phytohormone-induced signalling pathways and development in *Capsicum* plants has been studied using transcriptome sequencing analysis of *Capsicum* plants treated with four major phytohormones—SA, JA, Ethylene (ET) and ABA at six different time-points (Lee et al. 2020). Recently, an RNA seq dataset for as many as 78 RNA samples from *Capsicum* plants treated with heat, cold, salt and osmotic stress was published (Kang et al. 2020). The transcriptome data was verified using DEGs and GO enrichment analysis to generate a total of 204.68 Gb data.

The transcriptional dynamics in model plant Arabidopsis were analysed to unravel the plant responses to combinatorial stress conditions administered in a sequential manner (Coolen et al. 2016). The plants were subjected to biotic (fungal pathogen infection by *Botrytis cinerea* and herbivorous attack by larvae of *Pieris rapae*) and drought stress sequentially and the transcriptomic profiling of each kind of stress were obtained and compared with each other. The study concluded that the first stress response is pivotal for the identification of candidate genes in the stress response pathways and affects the level of tolerance during the subsequent stresses in ways similar to phytohormones. Gene families like *Receptor-like proteins* (*RLPs*) with roles in plant defense mechanisms have been characterised in Tomato genome (*Solanum lycopersicum* L.) (Kang and Yeom 2018). The *RLP* gene family is a large family of 176 genes in Tomato and were investigated for functional characterisation. The transcriptomic analysis of the leaf, bud, flower, fruit and root in tomato revealed that almost 71 RLP genes were highly expressed in at least one of the tissues while several RLPs displayed tissue-specific expression patterns. The tomato RLP genes were also studied for their role in disease resistance against bacteria and viruses in the same study.

Another group of plant specific transcription factors involved in phytohormone production and environmental stress responses are DNA-binding with one Zinc finger protein (Dofs). In pepper a total of 33 potential Dof factors have been identified and analysed (Kang et al. 2016). A major study and publication of two new pepper-reference genomes revealed the transposable element-aided expansion of nucleotide-binding and leucine-rich-repeat proteins (NLRs) in *Capsicum* plants (Kim et al. 2017a, b). *NLR* is a plant gene family with functional disease resistance genes. With more and more transcriptome data becoming available in *Capsicum*, the effects and responses to abiotic stresses at the level of gene expression can be studied. Transcriptomic profiling of fruit ripening stages in *Capsicum* were published for seven developmental stages of placenta and pericarp (Kim et al. 2018). This can serve as an important resource for further studies to understand abiotic stress mechanisms in *Capsicum* fruits. The multiple stress—heat, cold, salinity and osmotic stress treated *C. annuum* tissues were used for the transcriptomic profiling, identification and expression analysis of lncRNAs. Their potential roles in the regulation of stress responsive transcription factors like MYB, bZIP, WRKY etc. and interaction with miRNAs which eventually target the mRNAs was elucidated (Baruah et al. 2021).

3.7 Genetic Engineering for Resistance Traits

3.7.1 Genetic Transformation in Capsicum *for Abiotic Stress Resistance*

Capsicum transformation is difficult but not impossible. Regeneration is especially challenging but has been successful with modifications (Li et al. 2003). First attempt to obtain a transgenic *Capsicum* was done using Agrobacterium transformation in some wild accessions of Guatemalan cultivars and Yolo Wonder L. Buds elongation were achieved but shoot regeneration was unsuccessful (Liu et al. 1990). Successful transformation was achieved using Agrobacterium strain GV-3111 SE carrying a foreign gene, cucumber mosaic virus coat protein coat protein (CMV-CP) (Zhu et al. 1996) but again with unsuccessful shoot regeneration. Some attempts were made for anther culture for inheritance and expression analysis in hot pepper (Kim et al. 2007), but proper transformation and shoot regeneration still remains a challenge, which explains the plant's inflexibility as a model plant.

Capsicum genes have been used to express into plants sharing similar genomes like tomato or other members of the Solanaceae family and vice versa. Over time, progressively, with gene identifications, *Capsicum* was explored for ripening related functions, pungency biosynthesis and stable transformation protocols for both biotic and abiotic factors. Ripening genes viz. 1,4-beta-glucanase from pepper have been used to transform into tomato to study the polymerizing activity of cell walls (Harpster et al. 2002). Pepper *Bs2* gene identified by co-expression of *AvrBs2* conferred tolerance to tomato against *Xanthomonas campestrispv. Vesicatoria* (Tai et al. 1999). Pepper seeds were used to transform a rice MADS box transcription factor, *OsMADS1* to obtain a stable transformation and regeneration (Kim et al. 2001). A highly efficient pepper transformation system was conducted on *C. annuum* L. cotyledons using 4 genotypes, all showing high differentiation efficiency, elongation rate and rooting efficiency (Li et al. 2003). Constitutive expression of pepper xyloglucan endotransglucosylase homolog (*CaXTH3*) in models like Arabidopsis is shown to confer resistance to drought or freezing stress (Cho et al. 2006). Likewise, several abiotic stresses related factors have been described in each section of the abiotic stress portions in this book with their corresponding mitigations and quality control methods.

CaADIP1 (ABA and drought-induced protein phosphatase 1) localised in cytoplasm and nucleus was induced by both ABA and drought and negatively regulated ABA signalling (Lim and Lee 2016). The *CaADIP1-OX* Arabidopsis lines showed lesser sensitivity to ABA during germination and growth of seedling by reducing the expression of ABA-responsive *RAB18*, *RD20* and *RD29B* genes. Also, the overexpression of *CaADIP1* led to decreased stomatal closure, increased transpiration rate, and decreased tolerance to drought conditions than the wild type. On the contrary, the overexpression of ABA receptor protein *CaRLP1* (*C. annuum* RCAR-Like Protein 1) induced increased sensitivity to ABA treatment and increased tolerance to drought stress. The *CaADIP1-OX* and *CaRLP1-OX* double transgenic lines show that *CaRLP1* interacts with *CaADIP1* and inhibits its activity during seed germination in an ABA dependent manner.

TheY_1-SK_2 type dehydrin gene-*CaDHN3* from *C. annuum* was overexpressed in Arabidopsis to assess its function in salt and drought tolerance. The Arabidopsis OE lines demonstrated typical abiotic stress tolerance phenotypes like increased root length, chlorophyll content and rate of germination and decreased relative electrolyte leakage (REL) and MDA along with increased activities of antioxidant enzymes. Also the expression of four osmotic stress responsive genes—*DREB2A*, *ERD11*, *COR47* and *RD29B* was increased in *CaDHN3* of Arabidopsis plants. The alternate phenotypic and physiological characteristics were observed in *CaDHN3* silenced pepper plants (Meng et al. 2021).

3.7.2 Organelle Transformation

The accumulation of carotenoids in the chromoplast of the fruits was proved to be a constitutive process in several cultivars of Capsicums accompanied by xanthophyll esterification. This was confirmed by directly linking to the transformation of chromoplast and each component of the pigments was independent in their catabolism (Horero-Mendez et al. 2000). Transformation of a plastid lipid associated pepper fibrillin gene in tobacco resulted in co-localization of the proteins in stroma and thylakoid regions and is induced under drought and high light treatment (Rey et al. 2000).

3.7.3 Metabolic Engineering of Biosynthesis Pathways

Plant secondary metabolites are synthesized from a series of biosynthetic pathways. These biosynthetic pathways branch from its precursors and coalesce with other pathways catalyzed by several enzymes to synthesize complex macromolecules. Targeting rate limiting precursor enzymes, silencing competitive inhibitors and enhancing the expression of genes are hallmarks of metabolic engineering. In an early study targeting biosynthetic pathway genes, expression of cDNA clones of 3-hydroxy-3-methylglutaryl-coA reductase (*HMGR*), a rate limiting enzyme catalysing the synthesis of mevalonate, coordinately promoted the upregulation of defense related sesquiterpene phytoalexins in pepper against fungal infections in *C. annuum* L. cv. NocKwang (Ha et al. 2003). Even for the production of caffeic acid derived phenetyl esters and amides, acyl transferases from *C. annuum* were utilised with combinatorial effects from aromatic alcohol or amine biosynthesis in *E. coli* suggesting that genes such as caffeoylcoA methyl transferases (*cComT*) are potential candidates (Wang et al. 2017). Carotenoid biosynthetic genes viz. zeaxanthin epoxidase (*CaZEP*) in Capsicum have shown consequent production of violaxanthin (231ug/g dry weight) preceded by a RBS sequence in host organism *E. coli* (Takemura et al. 2019).

In fruits of *C. annuum* var. conoides, the lycopene cyclization genes were characterized to study the relationship between lycopene genes beta-lycopene (*CaLCYB1*, *CaLCYB2*, *CaLYCE1*) and capsorubin/capsanthin synthase (*CaCCS1*), inferring the competitive inhibition of *CaCCS1* by repression of *CaLCYE1* resulting in decreasing lutein content (Wang et al. 2019).

The most interesting trait in *Capsicum* or pepper plants is their pungency which is due to Capsaicinoids and dihydrocapsaicinoids. These compounds render the mammalian intolerance property of the fruits. They accumulate in the vacuoles and vesicles of the epidermal cells of placental tissue of the fruits and are differentially expressed at different developmental phases of ripening (Bosland et al. 2015). Their synthesis is governed by a series of enzymes from two biosynthetic pathways, phenylpropanoid and fatty acid biosynthesis pathway (Aza-Gonzalez et al. 2011). Some of these genes are individually characterized and mapped but their regulation and signalling networks remain underexplored due to their limited dynamics and reproducibility of transformation and transgenic experiments. The locus that code for the Capsaicin synthase was named *Pun1* situated at chromosome 2 (Stewart et al. 2005). Genes *PAL*, *Ca4H*, *CCoAOMT*, *Kas* and *pAMT* correspond to the levels of pungency and are differentially expressed across tissues at various fruit developmental and ripening stages (Zhang et al. 2016). Silencing of these genes reduced the levels of pungency (Abraham-Juárez et al. 2008). Thus, these genes are significant targets for the improved production of chilli or the compound attributes. In the recent decade, de novo and transcriptomics sequencing (Kim et al. 2014b; Qin et al. 2014) have opened the curtain for the revelation of a plethora of regulatory networks, transcripts and proteins for future research in this unique and interesting plant.

3.8 Gene and Genome Databases

Bioinformatics plays an important role in stress related research, as nature of most biological explorations are highly data driven and attains great complexities in experimental designs as well. Few databases of genetic and genomic resources have been reported for *Capsicum* species. These databases helps data accessibility through web servers such as Pepper Genome Database (PGD; http://pgd.pepper.snu.ac.kr/index.php?a=view) for assessing genome sequence information, Pepper EST Database (http://genepool.kribb.re.kr/pepper) for transcriptomic analysis, EnsemBl Plants browser for *C. annuum* database (https://plants.ensembl.org/Capsicum_annuum/Info/Index), and TGsol database for comparative analysis with other members of the Solanaceae which also allows for syntenic identification through homologous screening (http://tgsol.seeders.co.kr/scomp/). Other databases like European Cooperative Programme for Plant Genetic Resources (ECPGR; https://www.ecpgr.cgiar.org/working-groups/solanaceae) are not specific to pepper and Solanaceae but provide access to pepper germplasm information along with the phenotypic information for pepper varieties, across Europe. Softwares like Carthagene are efficient for the linkage analysis of molecular markers (de Givry et al. 2005). Gene prediction web tools like FGENESH (http://linux1.softberry.Com), blastx and blastp are used to determine the genes in the linkage group. Using the predicted amino acid sequences, these programs help in searching already annotated genes and homologous sequences. Some mainstream databases viz.

KEGG databases and SolCyc (https://solcyc.solgenomics.net/CAP/NEW-IMAGE?type=OVERVIEW&force=t) from Sol genomics also provide information on the integration of metabolic and biosynthetic pathways. The assembled genome information, homologous gene search features and genome structure are also available on public databases such as NCBI and PepperHub.

3.9 Conclusion and Future Perspectives

The *Capsicum* crop has faced massive losses in yield, production and quality due to the myriad of abiotic stresses and environmental factors, in solitary and multitude, in the past decades. For the management of abiotic stress-induced crop losses, there is an extensive need for identification and characterisation of the genetic and molecular mechanisms of the plant defence system, in addition to the already existing reservoir of plant genetic resources. More studies focusing on genetic mapping, transcriptomic and expression analysis are required for a comprehensive understanding of stress-related response mechanisms during variable abiotic stress conditions and environmental signalling, in order to develop an integrated approach for dealing with unprecedented weather conditions. Also, the systematic examination of *Capsicum* germplasm on a global level for identifying sources of tolerance and resistance would greatly contribute to the overall management and prevention of abiotic stress related complexities in *Capsicum.* Ultimately, in place of conventional practices and methodologies, advanced strategies like multi parental population like NAM (nested association mapping), and MAGIC (multi parent advanced generation intercross) should be adopted and used. Improvement of *Capsicum* varieties for efficient production in order to fulfill the increasing world-wide demand and thereby increasing the financial returns of the farmers should be the ultimate goal. The sustainable management of environmental stresses is necessary keeping in mind the limited resources and their conservation.

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Chapter 4
Next Generation Breeding for Abiotic Stress Resistance in Eggplant

Laura Toppino, Lorenzo Barchi, and Giuseppe Leonardo Rotino

Abstract Eggplant (*Solanum melongena* L.) is a worldwide cultivated vegetable crop from the Solanaceae family whose berries are an important component of human's daily diet and are rich in health-related compounds. As it happens with many domesticates, this species is sensitive to many abiotic stresses including drought, extreme temperatures, salinity, and soil toxicity due to heavy metals, which make tackling the worsening environmental conditions provoked by the upcoming climate changes more challenging. The natural diversity available in cultivated eggplant for agroclimatic adaptation is wide but still poorly explored. Moreover, many wild allied relatives of this crop may play key role as source of traits for adaptation to adverse conditions. To this purpose, the identification of sources of tolerance to the main stresses is a first step for conventional or marker-assisted breeding for these traits. Indeed, throughout the last decades, multiple screenings involving both intraspecific and interspecific genetic resources have been performed, thus paving the way to the selection of varieties with a greater degree of tolerance to the most important abiotic stresses. The recent availability of completely anchored and annotated genome sequences of eggplant provided an opportunity for carrying on comparative analysis at the transcriptome level in eggplant. Great efforts have been focused in elucidating the molecular pathways and gene expression networks affected in eggplant and some wild relatives under different abiotic stresses. In the present chapter, the description of the main abiotic stresses affecting eggplant, as well as of the available genetic resources exploitable as source of adaptation traits to adverse conditions are reported. Furthermore, an up-to-date inventory of the employment of allied and wild relatives in breeding programs carried out in the last decades aimed at improving the crop's adaptability to adverse conditions is described. Finally, the identification of QTLs and genes underlying traits of interest, the genomics efforts

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carried out within the species are comprehensively discussed, as well as their usefulness in acquiring deeper knowledge of the genetic, physiological and biochemical mechanisms involved in tolerance to abiotic stresses.

Keywords *Solanum* · Wild species · Abiotic stresses · Breeding resources · Quantitative trait loci · Genetic maps · Genome sequence

4.1 Introduction

Eggplant (*Solanum melongena* L.) known as aubergine is a crop of the Solanaceae family and an important vegetable cultivated on more than 1.86 million hectares in tropical and subtropical regions (Rotino et al. 2014), especially in Asia and Mediterranean basin. The global production surpassed 54 million tons in 2019, with China, India, Egypt, Turkey, and Iran among the top five eggplant producing countries (Faostat 2021). Food shortages could arise more frequently, owing to unpredictable crop yield losses caused by biotic and abiotic stresses, and as occurs with many domesticates, common eggplant is susceptible to several abiotic stresses, including drought, salinity, low and high temperatures, and soil toxicity (Savvas and Lenz 2000; Boyaci et al. 2009; Brand-Daunay and Hazra 2012; Wu et al. 2014b; Díaz-Pérez and Eaton 2015; Plazas et al. 2019).

To better address the challenges posed by adaptation to the climate change, innovative tools for breeding, as well as of a deeper knowledge of the genetic, physiological and biochemical mechanisms involved in tolerance to abiotic stresses, are getting more and more important to develop new breeding materials able to speedily recover from biotic and abiotic stresses. This will be achieved by developing, from the one hand improved crop varieties to enhance genetic diversity and from the other hand by identifying markers, quantitative trait loci (QTLs) and candidate genes involved in the response to abiotic stresses as also tailoring microbial communities which have biocontrol and/or biostimulant functionality. Throughout the last decades multiple screenings for some of the most important stresses have been performed, involving both intraspecific and interspecific genetic resources (Daunay 2008; Toppino et al. 2020). The natural diversity available for agroclimatic adaptation in cultivated eggplant is wide and it is being already evaluated in response to several biotic and abiotic stresses. Furthermore, crop wild relatives (CWRs), are an important source of potential genetic variability for many relevant traits which could represent a source for widening the genetic background of the cultivated *S. melongena* and creating a new generation of breeding lines and cultivars with dramatically increased tolerance to adverse environmental conditions.

Next generation sequencing (NGS) technologies allow to develop high quality genome sequences for many crops including eggplant (Hirakawa et al. 2014; Barchi et al. 2019a, b, 2021). Furthermore, RNA-seq experiments allow to capture the transcriptional changes in contrasting accessions for stress resistance/tolerance and to highlight the involvement of specific gene families and identification of the key genes.

Thanks to high-throughput genotyping technology, large-scale association analyses such as genome-wide association studies (GWAS) were performed in eggplant (Cericola et al. 2014; Portis et al. 2015), allowing to dissect the genetic architecture of many different traits of breeding interest.

4.2 Abiotic Stresses Affecting Eggplant

High temperature and soil salinization severely affect crop yield especially of those cultivation systems dependent from irrigation as in eggplant; therefore, extensive research is needed to evaluate the eggplant responses to abiotic stresses, and particularly to the climate change-induced ones, both at physiological and metabolic level.

4.2.1 Salt

Eggplant is moderately susceptible to salinity when compared to other Solanaceae like pepper (Ünlükara et al. 2010; Shahbaz et al. 2012). Salinity has a negative impact on plant growth, fruit quality and yield (Savvas and Lenz 1996, 2000; Yasar et al. 2006; Hanachi et al. 2014; Hannachi and Van Labeke 2018) and may also induce a combination of unfavourable effects on sensitive plants including osmotic stress, ion toxicity and oxidative stress. Extensive research has been carried out to examine salt-induced morphological, biochemical, and physiological changes in eggplant (Chartzoulakis and Loupassaki 1997; Hamdy et al. 2002; Akinci et al. 2004; Saeed et al. 2014; Hanachi et al. 2014). Different studies reported that salt-induced growth reduction in eggplant plants is associated with high accumulation of Na^+ and Cl^- in both roots and shoots (Hasegawa et al. 1986; Akinci et al. 2004; Unlukara et al. 2010) which causes stomata closure (osmotic stress) and increase leaf turgor potential, entailing that this plant, being a glycophyte, is unable to manage the uptake of Na^+ under high salinity (Akinci et al. 2004). In contrast, water use efficiency of eggplant is not affected by salt stress (Abbas et al. 2010). It has been observed that water consumption, as well as Potassium (K^+), Calcium (Ca^{2+}) concentrations and the K^+/Na^+ ratio decrease (Unlukara et al. 2010; et al. 2019) while glycinebetaine and proline levels increase in plant tissues under saline conditions. Overall, salinity stress by excess of NaCl [to a lesser extent also by $Ca(NO_3)_2$] is reported to harshly reduce the seed germination ability (Saeed et al. 2014), the growth of root and shoot, the chlorophyll content and the photosynthetic rates, as well as determine a drop in fruit yield (Abbas et al. 2010). A rising of free proline and leaf glycinebetaine (GB), malondialdehyde (MDA) and H_2O_2 contents was observed under varying saline regimes (Shaheen et al. 2013), suggesting the role of protective mechanisms of antioxidant enzymes and polyamines. The induction of antioxidant enzymes such as catalase and peroxidase can be considered as one mechanism of salt tolerance in plants as salinity significantly decreased superoxide dismutase (SOD) and increased peroxidase (POD) activities, while not affecting catalase (CAT) activities.

4.2.2 Heat

With increased global warming, the climate is gradually becoming warmer. Temperatures of 38 °C even as high as 43 °C in the summer can seriously inhibit growth of seedling, flower development, fruit set and growth thus affecting yield and, eventually, impact the fruit quality. Eggplant is a thermophilic plant, known to have a better tolerance against supra-optimal soil temperatures than tomato. Notwithstanding, temperatures close to or above 40ºC may prevent growth, pollen production and viability, moreover, can provoke flower abortion and reduce yield and fruit quality (Wu et al. 2014b). High temperature also is responsible for a significant decrease in the total anthocyanin content in peels and in the lightening of the peel color (Li et al. 2018; Lv et al. 2019; Zhang et al. 2019, 2020; Wu et al. 2020) as fasten the fruit ripening process. Studies have been carried out hence for evaluating eggplant as rootstocks for tomato scions (Abdelmageed and Gruda 2009), while grafted eggplant onto heat-tolerant eggplant rootstocks resulted in a prolonged growth stage and an increased yield of up to 10% (Wang et al. 2006).

4.2.3 Drought

Drought stress represents one of the most important environmental stresses that can negatively influence soil physiochemical and biological properties, as well as the growth and productivity of crops including eggplant (Fu et al. 2013) by reducing growth, development, and yield of crops (Fisher 1980). In many regions, in arid and semi-arid conditions particularly, drought stress is generally associated with high soil salinity mainly due to high evaporation (Senyigit et al. 2011; Souri et al. 2019). It also causes an increment in catalase, carotenoid and proline content resulting in a higher antioxidant activity (Reddy et al. 2004). Drought is known to affect 40–60% of the world's total agricultural lands, and, given that many areas of cultivation of eggplant may become prone to drought in the near future, there is a need for drought-tolerant improved varieties of eggplant. As occurs in many crops, drought stress is responsible of the reduction of growth and yield in eggplant, although this species seems more tolerant if compared to the other vegetables of the same family like tomato and pepper (Díaz-Pérez and Eaton 2015). Plants grown under high water stress have a reduced growth and produce a lower number of fruits with poorer quality with respect to plants cultivated in normal conditions (Kirnak et al. 2002). Consequently, a reduction in both relative leaf expansion rate (RLER) and transpiration (Kirnak et al. 2001) is evidenced. Water scarcity also negatively affects the uptake of Nitrogen, Phosphorus and Potassium and severely decreases content of total soluble solids (TSS), proteins and carotenoids in the fruit while, on the contrary, it causes an increased amount of total phenols, superoxide dismutase (SOD), glutathione reductase (GR), proline, electrolyte leakage, pH and vitamin C (Rodan et al. 2020). A detailed study on four diverse eggplant genotypes allowed to identify a reduction of the photosynthetic pigments, an increase of proline, malondialdehyde, total phenolics, and total flavonoids as the main effects associated to the water stress, and proline was suggested as a marker for drought stress tolerance in eggplant (Plazas et al. 2019).

4.2.4 Cold

In recent years, due to frequent occurrence of low temperature in the early stage of cultivation, cold stress is to take into consideration because it limits plant growth, development, and production. Compared with other Solanaceous vegetables, eggplant is much more sensitive to low temperatures (Wan et al. 2014). Eggplant grows slowly when the temperature is below 17 °C, suffers rapid physiological disorders below 10 °C and undergoes chilling injury near 7.2 °C (Zhang et al. 2000). Chilling Injury (CI) is a physiological disorder, whose main symptoms are rapid aging, skin shrinkage, calyx deterioration and browning (Fallik et al. 1995). Growing eggplants at low temperatures causes abnormal growth with disordered metabolism and chilling damage (Concellon et al. 2005, 2007), as well as reduction of pollen viability (Daunay and Hazra 2012), resulting in decreased fruit set due to absence of pollination and fertilization (Donzella et al. 2000); in some growing area this phenomenon occurs every early spring causing serious losses (Wan et al. 2014). Unfortunately, the mechanism of molecular response of the species to cold stress still remains to be elucidated. The exploitation of the intraspecific variability available has allowed the selection and development of parthenocarpic cultivars which allow fruit set under low temperatures that reduce pollen viability (Kikuchi et al. 2008). Improved tolerance to low temperature would be also important for the unheated protected cultivation of eggplant considering also that often there is a strong excursion between night and day temperatures.

Temperature is also important to maintain the quality of eggplant after harvesting. Postharvest storage of eggplants at room temperature rapidly reduces the quality of the fruit, mainly due to water loss, shrinkage of the skin, and discoloration; thus, cold storage is generally used to maintain postharvest fruit quality. However, eggplants are cold-sensitive and prone to chilling injury (CI) when stored at temperatures <12 °C (Concellón et al. 2005). Regarding reactive oxygen species (ROS) scavengers, the activity of antioxidant enzymes in eggplant fruit can be decreased by low temperature (Zheng et al. 2008). The incidence of CI may be reduced using several techniques, including optimized storage conditioning (Abe and Chachin 1985) or a fluctuating storage temperature (Ito and Nakamura 1984). Application of a low temperature conditioning (LTC), a short-term storage of cold-sensitive plants above the damage-threshold temperature, is reported in eggplant to stimulate tolerance to subsequent storage at lower temperatures as it significantly reduces the severity of CI and maintains product quality during low-temperature storage in eggplant (Shi et al. 2018).

4.2.5 Heavy Metals

It is well known that high concentrations of heavy metals like Cadmium, Chromium, Lead, and Nickel in the soil may have a toxic effect for eggplant plants (Pandey et al. 2010; Yuan et al. 2019). Recent studies suggest that safety of eggplants consumption from polluted farmlands has suffered because of the elevated levels of heavy metals such as Pb and Cd accumulated in eggplant (Saeedifar et al. 2014; Ye et al. 2015). Concentrations of heavy metals in eggplant and soil decreases from upstream (pollution source) to downstream in a zonal area along a polluted stream. In addition, long-term wastewater irrigation increases the heavy metal concentrations in soils and plants reducing the yields of eggplants and having an impact on their accumulations in the fruits. Content of heavy metals in eggplant tissues may be predicted, using a linear model, by measuring their corresponding heavy metals in soils (Ai et al. 2018). Eggplant should not be cultivated in the farms and fields which use sewage sludge contaminated with heavy metals as an amendment, as this application causes a significant accumulation of Pb, Cd and Ni metals in all tissues but mostly in the fruits (Youssef and Abd El-Gawad 2018) which become not safe for the human consumption as exceeding the permissible limits. Soil toxicity effects have been barely studied in eggplant so far, and little is known on intraspecific variation existing for tolerance trait to heavy metals. However, it has been found that grafting eggplant onto its wild relative *S. torvum* improves tolerance to Cd toxicity by reducing its translocation to the aerial parts (Yamaguchi et al. 2011; Yuan et al. 2019). In contrast to *S. torvum*, *S. nigrum* is a Cadmium (Cd) accumulator. To decipher the molecular mechanisms that are responsible for differential cadmium (Cd) accumulation in the two *Solanum* species, Xu et al. (2012) carried out a comparative transcriptome analysis. Results revealed a higher expression of the genes that encode several metal transporters as well as antioxidant-related genes, and several organic and amino acid biosynthesis/metabolism-related genes in Cd-treated *S. nigrum*. Overall, information obtained provided an insight into novel strategies that may be used for phytoremediation and food safety.

4.2.6 Low Nitrate

Eggplant yield is sensitive to nitrogen fertilization. However, its excessive use, especially in the more intensive cultivation system, is responsible for environmental damage and, consequently, negatively affect human health. Nitrogen fertilization affects the growth and yield of eggplant in terms of plant vigour, leaf chlorophyll content, fruit settings, dry matter production and ascorbic acid content (Rosati et al. 2001) and may also affect NaCl toxicity (Singh et al. 2016). Akanbi et al. (2007) reported that nitrogen fertilization affects flower number, fruit pH, total solid content, fruit weight and seed number. Plant height, lateral stem number, leaf chlorophyll content, flower number are all significantly improved in plants supplied with over-recommended values of nitrogen fertilization (Wange and kale 2004), and eggplant fruit girth, weight and yield increase along with the increment of N fertilization (Devi et al. 2002; Aminifard et al. 2010), but exceeding values up to 190 kg/ha of N significantly delay flowering and fruit setting (Pal et al. 2002, 2003). Amiri et al. (2012), also tested the combined effect in eggplant of irrigation intervals and nitrogen fertilization in dry land on the WUE, yield, and yield parameters. The selection of improved nitrogen-use-efficiency (NUE) genotypes, more able to uptake, translocate and utilize N available in soils, would be challenging to maintain high yields in a sustainable agriculture with a lower input. Despite the relevance and the complexity of plant NUE, essential for the development of a sustainable agriculture, limited information on genetic variation for this trait are available for eggplant, whose productivity is highly sensitive to N fertilizer (Pal et al. 2002).

4.3 Germplasm and Gene Pools Available as Sources of Resistance to Abiotic Stresses

4.3.1 Exploitation of Natural Diversity in Common Eggplant

The natural diversity available for agroclimatic adaptation in the gene pool of the common eggplant is quite wide, covering fast to slow growth in cold or warm or hot conditions, short to long fruiting period, early to late fruiting ability, weak to vigorous vegetation, short to long internodes, erected to prostrate growth habit, etc. (Daunay et al. 2008). Some germplasm characterizations have been performed for the identification of sources of tolerance to abiotic stresses such as drought, extreme temperatures and, especially, salinity.

A significant within species variation in respect to salt tolerance was already depicted (Hamdy et al. 2002; Akinci et al. 2004, Abbas et al. 2010; Mustafa et al. 2017; Hannachi and Van Labeke 2018), providing useful information exploitable to select varieties with a higher degree of tolerance. Recently, a wide phenotypic variability was highlighted among 50 eggplant genotypes, including commercial varieties, local cultivars and breeding lines with the aim to identify genetically divergent genotypes for fruit yield and its component characters under saline stress condition (Thirumalai et al. 2020).

Differences between different accessions of cultivated eggplant were found for tolerance to drought or salinity (Hamdy et al. 2002; Ahire and Nikam 2011; Bhatt et al. 2014; Hanachi et al. 2014; Saracanlao et al. 2016; Zayova et al. 2017; Kiran et al. 2019; Plazas et al. 2019; Kouassi et al. 2021; Thant et al. 2021), also combining different stresses and evaluating the response to fungal infection in relation to different environmental conditions (Tani et al. 2018).

Root vigour, stomatal density, stomatal resistance to water vapor diffusion, showed a role in the ability of the varieties to grow and set fruits in dry or humid climates (Kouassi et al. 2021). Differences among eggplant genotypes have been found for tolerance to low temperatures (Abak and Guler 1993; Boyaci et al. 2009; Yang et al. 2020a)~~,~~ as well as for heat stress (Santhiya et al. 2019), with several promising materials identified opening the way for the development of more tolerant cultivars.

Finally, genotypes with improved NUE have been identified (Mauceri et al. 2020) evidencing that the genetic diversity for NUE is mainly attributable to the Nitrogen utilization component of NUE and, most recently, the gene networks responsible such diversity recognized (Mauceri et al. 2021).

4.3.2 Sources of Tolerance in Allied and Wild Relatives

Crop wild relatives (CWRs) of eggplant are an important source of potential variability and allelic variation for the genetic traits controlling several agronomic and qualitative features of plant and fruit (Rotino et al. 2014; Kaushik et al. 2016; Dempewolf et al. 2014; Chapman 2020; Daunay et al. 2019; Toppino et al. 2020). Although variation at genome level exists within eggplant gene pool (Behera et al. 2006; Liu et al. 2018), the genetic diversity of its wild and allied relatives is much higher (Vorontsova et al. 2013). Thus, CWRs represent a valuable source for enlarging the genetic background of the cultivated *S. melongena* for facing the challenge of adaptation to the climate change.

Eggplant CWRs grow in a wide range of environmental conditions, including dry, semi desertic and desertic areas as well as under highly stressful environments (Knapp et al. 2013, 2019; Syfert et al. 2016; Vorontsova and Knapp 2016; Ranil et al. 2017). Some of CWRs have been found to be resistant or tolerant to several prevailing diseases and insect pests (Daunay and Hazra 2012), also representing an important source of variation for introgression of abiotic stress tolerance in eggplant (Prohens et al. 2017).

Several CWRs of eggplant are reported to be tolerant to drought, as they naturally grow in areas where water availability is minimal (Knapp et al. 2013, 2017; Syfert et al. 2016; Vorontsova and Knapp 2016). To this regard, some accessions tolerant to drought of *S. insanum*, the sexually compatible progenitor of *S. melongena*, (Ranil et al. 2017) can be quickly incorporated in breeding programs (Kouassi et al. 2016). *Solanum incanum*, an eggplant sister species which grows in desertic and semi-desertic areas, is exposed to very high temperatures during the summer season. Thus, it has been identified as highly promising for breeding for tolerance to drought and employed in crosses with *S. melongena* to obtain lines with improved tolerance to drought (Knapp et al. 2013; Vorontsova et al. 2016; Gramazio et al. 2017; Plazas et al. 2020a, b; Kouassi et al. 2021).

Within the secondary gene pool, *S. macrocarpon* and *S. linnaeanum* have been described as tolerant to drought (Daunay et al. 1991). The scarlet eggplant (*Solanum aethiopicum* L.), belonging to the Anguivi grade, is divided into four major groups: “Gilo," “Shum," “Kumba,” and “Aculeatum”. Previous studies showed different response to abiotic stresses of the accessions (Mibei et al. 2017; Sseremba et al. 2018a, b), while analysis of domestication and demographic history revealed, among the four major groups, active selection for genes involved in drought as well as salt tolerance in both “Gilo” and “Shum” groups (Song et al. 2019); moreover, *S. incanum* together with other species is considered very promising for improving drought tolerance in eggplant (Knapp et al. 2013; Gramazio et al. 2017). Within the tertiary genepool, *S. elaeagnifolium* was found to be particularly tolerant to drought (Fita et al. 2015; Knapp et al. 2017) due to a more expanded root system with respect to that of *S. melongena.*

Solanum torvum is one of the mostly studied eggplant wild relative as it displays, other than resistance to soil-borne diseases, a high tolerance to salinity and also proved to limit the translocation of Cadmium in the shoot (Yamaguchi et al. 2011; Yuan et al. 2019), thus it was proposed for soil phytoremediation (Mori et al. 2009) together with *Solanum nigrum* (Xu et al. 2012).

S. insanum (Brenes et al. 2020a), *S. torvum* (Chen et al. 2012; Brenes et al. 2020b), *S. linnaeanum* (Zhuang et al. 2014), and *S. aethiopicum* were reported as tolerant to salt and displayed a higher level of proline, Na^+ and Cl^- accumulation than eggplant (Ranil et al. 2017; Brenes et al. 2020a, 2020b).

Some CWRs such as *S. aculeatissimum*, *S. mammosum*, and *S. grandiflorum* have been described as tolerant to low temperatures (Rotino et al. 2014; Yang et al. 2017) and could be of interest for eggplant breeding.

It is worth to point out that some precautions must be taken when the allied and wild species are considered for breeding purposes. Indeed, it would be expected that CWRs display an even wider allelic variation for the useful traits in study, because they have been mainly subjected to environmental selection including the indirect effect of the human activities and not to the bottleneck of domestication (Toppino et al. 2020). Moreover, despite their value and usefulness for genetic improvement of eggplant, some of them may carry undesirable traits, such as the very high susceptibility to *Colletotrichum gloeosporioides* found in *S. torvum*, and the bitter taste, due to a high level of steroid saponin in *S. Linnaeanum* (Collonnier et al. 2001a, b). However, in some cases introgressed progenies have been obtained by crossing eggplant with wild relatives which could be exploited for highlighting traits of tolerance to many abiotic stresses, like drought.

4.4 Grafting

Grafting proved a useful technique to increase yields, overcome diseases, alter growth habit, and improve stress tolerance because of the vigorous root system of rootstocks (Lee 1994; Soriano et al. 2003; Bletsos 2006; López-Gómez et al. 2007; Colla et al. 2010; Gao et al. 2016; Boyaci and Ellialtioglu 2018; Elliantlogu et al. 2019). Grafting may also affect oxidative damages, polyamines content and metabolism and antioxidant enzymes activities allowing better performance of eggplant plants under $Ca(NO_3)_2$ stress (Wei et al. 2009). The direct use of wild species as rootstocks may be a direct way to use CWRs for alleviating salt injury stresses and enhancing photosynthesis, root activity and fruit yield, as demonstrated for *S. torvum* (Bletsos et al. 2003; Colla et al. 2010; Zhou et al. 2010). Moreover, the plant height, stem diameter, fresh weight of vegetative parts and roots, root growth, as well as chlorophyll and proline content and peroxidase (POD), polyphenoloxidase (PPO), and phenylalanine ammonia-lyase (PAL) activities of grafted eggplants were higher than in self-rooted eggplant controls (Bai et al. 2005, 2009) under stressed conditions. Qian et al. (2013) demonstrated that the yield of grafted eggplant onto *S. torvum* was significantly higher than that of the non-grafted one under seawater salinity stress. Along with an increase in salt concentration, grafted eggplant increased the content of N and P, could still maintain a balanced absorption of Ca, Mg, Cu, Fe, Zn and Mn while the K content and the ratio of Na^+/K^+ decreased significantly. The use of S. *torvum* rootstocks for eggplant grafting was further reviewed by Li et al. (2013); thus, this species continues to be a potential source for new research on rootstock. Grafted eggplant seedlings, using salinity tolerant commercial *S. torvum* Swartz as rootstock, were more tolerant to stress by excess of $Ca(NO_3)_2$ than non-grafted seedlings, owing to the effectively scavenging system of free radicals and to the protective mechanisms of antioxidant enzymes and polyamines (Wei et al. 2009).

A detailed characterization of the mechanism of Cadmium (Cd) translocation in *S. torvum* with respect to cultivated eggplant revealed that although they displayed a similar rate of adsorption in the roots, the wild species showed a significant lower translocation of Cd in the stem, leaf, and fruit and, consequently, Cd concentration in the fruits was reported drastically reduced by grafting onto *S. torvum* (Yamaguchi et al. 2011; Yuan et al. 2019).

The most interesting rootstock traits, especially regarding abiotic stresses, are quite complex and regulated by multiple genes (Keatinge et al. 2014) and further influenced by the physiological and molecular cross-talks between scion and rootstock, therefore breeding and evaluation of appropriate rootstocks is still quite a challenging activity.

4.5 Symptom's Alleviation Through Ectopic Application

Exogenous application of inorganic fertilizers, compatible solutes, and plant growth promoting bacteria have been found to be viable approaches to enhance salt tolerance (Abbas et al. 2010; Elwan 2010; Wu et al. 2014a). For example, Elwan (2010) reported that foliar-applied di-potassium hydrogen orthophosphate (K_2HPO_4) ameliorated the adverse effects of NaCl on plant growth and development, crop fruit yield, and total soluble sugar contents. Foliar application of K_2HPO_4 did not affect Na^+ uptake in plants but its concentration was reduced in fruits of salt-stressed plants treated with fertilizer. However, it increased leaf K^+ and Ca^{2+} content under saline conditions (Elwan 2010). As the salt tolerance mechanism in eggplant is quite explicitly elucidated, it may be possible to minimize its negative effect: Abbas et al. (2010) proved that the alleviation of salt-induced negative effects may be achieved by foliar application of glycinbetaine (GB) or sugar beet extracts which have a positive effect on growth and other important physiological processes in eggplant. Moreover, sugar beet extract proved to be a cheaper alternative source of natural GB even more effective than the pure molecule. Different levels of N significantly modulated NaCl-induced damaging effects in eggplant. After N addition, Na^+ content, enzymatic antioxidants, and pool of free proline and activity of pyrroline-5-carboxylate synthase are finely regulated, which might be associated with the mitigation of NaCl stress, and this effect was more pronounced with supra-optimum levels of N (Singh et al. 2016). Another strategy to enhance eggplant salt tolerance is inoculation with plant growth promoting bacteria (Fu et al. 2010). For example, seeds of eggplant inoculated with the rhizobacterium *Pseudomonas* sp. *DW1* showed improved germination under saline conditions. Inoculation with bacteria also enhanced the shoot Ca^{2+} content but had no effect on Na^+ shoot content. Activity of superoxide dismutase, an antioxidant enzyme, was also increased by inoculation with rhizobacteria. Both processes, i.e., alteration in mineral nutrients and antioxidant activities, were found to be effectively involved in enhancing eggplant salt tolerance (Fu et al. 2010). The exogenous application of 24-epibrassinolide resulted in a protective effect on growth and physiology of eggplant under salt stress due to a better antioxidant activity for the effective removal of ROS and maintenance of homeostasis (Ding et al. 2012).

Pre-treatment with salicylic acid (SA) is reported to have a positive effect on eggplant chilling resistance of eggplant seedlings: the maximum level of improvement, corresponding to more than 30% decrement of chilling injury being obtained with a SA pre-treatment concentration of 0.3% as compared to untreated seedlings. Under chilling stress, seedlings pre-treated with SA displayed lower hydrogen peroxide (H_2O_2) content and higher ascorbic acid and ascorbate–glutathione (GSH) content and increased ascorbate peroxidase (APX) activity (Chen et al. 2011) than the controls.

The postharvest chilling injury of eggplant fruits was significantly reduced by Eugenol (EUG) fumigation which effectively retarded the symptoms development, reduced flesh browning, weight loss, and malondialdehyde content, and sustained soluble solids content and proline content; moreover, EUG fumigation inhibited the activities of polyphenol oxidase (PPO) and peroxidase (POD) (Huang et al. 2019).

Technical management of the cultivation significantly affect the response of eggplant to water deficit and heat stress. As examples, the use of vermicompost and biochar increased eggplant vegetative growth, yield and water use efficiency (WUE) under water deficit conditions (Abd El-Mageed et al. 2021a; Ebrahimi et al. 2021). Also, the application of the mulches is recommended for eggplant in areas affected by water shortages, (Sabatino et al. 2018) and it is reported that both the application of hay and/or plastic mulch alleviated the deleterious effects of water deficit (Rodan et al. 2020, 2021). Foliar spraying of ZnO nanoparticles gives the utility for alleviating drought stress effects on eggplant cultivated in saline soil (Abd

El-Mageed et al. 2021b; Semida et al. 2021). Arbuscular mycorrhizal fungi significantly improved the WUE and uptake of fertilizer under reduced watering ensuring a higher yield with respect to the non-inoculated eggplant (Badr et al. 2020). Identification of genotypes and superior alleles which allows to further maximize these positive effects may contribute to a better resilience of eggplant cultivation system for facing the challenge of abiotic stress resistance.

4.6 Introgression Breeding Through Interspecific Hybridization: Methods and Achievements

Introgression breeding in eggplant landraces from areas particularly sensitive to climate change using a broad range of eggplant CWRs could contribute to mitigate the impact of climate change in the production of this vegetable crop (Prohens et al. 2017). Many interspecific hybrids were obtained in recent years, advanced backcross materials and introgression lines with multiple eggplant CWRs which are highly promising for broadening the gene pool of eggplant and for adapting this crop to climate change as they display enhanced performance under adverse environmental conditions.

Eggplant is one of the vegetable crops in which significant efforts are being done in the last years for introgression breeding from related species for adaptation to climate change (Liu et al. 2015; Kouassi et al. 2016; Plazas et al. 2016, 2020a, b; Toppino et al. 2019; Boyaci et al. 2020),

To evaluate the impact of interspecific hybridization on drought tolerance 12 hybrids between nine accessions of eggplant and seven related species (of which six are CWRs and one is a cultivated relative), data on vegetative growth and yield-and related traits collected during the rainy and dry seasons revealed that the F_1 hybrid progenies of eggplant crossed with *S. insanum, S. anguivi,* and *S. dasyphyllum*, plus the *S. sysimbriifolium* accession SIS1, displayed improved drought tolerance (Kouassi et al. 2021).

Recently, multiple crosses were performed between eggplant and 15 CWRs (Plazas et al. 2020a, b). A total of 90 hybrid combinations were obtained, including 48 first backcross (BC1) and 36 s backcross (BC2) generations. The hybrids were generated by crossing six eggplant landraces with the primary gene pool species *S. insanum*, eight secondary gene pool species (*S. anguivi, S. dasyphyllum, S. incanum, S. lichtensteinii, S. lidii, S. linnaeanum, S. pyracanthos,* and *S. tomentosum*), as well as the tertiary gene pool species *S. elaeagnifolium,* which is a drought tolerant invasive weed and thus it is of particular interest for improving the tolerance to drought in eggplant, particularly because backcrosses of the interspecific hybrid with eggplant have been obtained (García-Fortea et al. 2019). In addition, single seed descent progenies from individually selected BC_2 plants and deriving from the use as wild parent *S. anguivi, S. dasyphyllum, S. incanum, S. insanum, S. lichtensteinii* and *S. lidi*i were also obtained. Backcross progenies (BC_1) derived from the crossing between

S. incanum and eggplant have been used to construct an interspecific linkage map and develop introgression lines with improved tolerance to abiotic stresses (Vilanova et al. 2010; Prohens et al. 2012, 2013). After several generations of backcross and marker-assisted selection, the first set of fixed introgression lines containing single introgressions of *S. incanum* has been developed (Gramazio et al. 2017).

Moreover, advanced introgression breeding lines from sexual crosses of eggplant with *S. linneanum* (Acciarri et al. 2004; Liu et al. 2015) and from somatic hybrids with *S. aethiopicum* (Rizza et al. 2002) and *S. integrifolium* (Rotino et al. 1998), initially employed for the introgression of resistance traits to *Verticillium dahliae* and *Fusarium oxysporum* (Rotino 1998; Rizza 2002; Toppino et al. 2008a, b) into the genetic background of eggplant, could also be evaluated for their potential tolerance to abiotic stresses and exploited in breeding programs in particular for drought and salt resistance.

4.7 In Vitro Culture Approaches for Increasing Genetic Diversity

Many attempts have been made to introgress key traits for breeding from wild species to cultivated varieties of eggplant, with limited success so far and generally due to existing sexual barriers between eggplant and its allied species (Rao 1979; Rotino et al. 2014; Plazas et al. 2016; Boyaci et al. 2020; Toppino et al. 2021).

Although some interspecific hybrids were obtained through sexual crosses between eggplant and nearly 30 CWRs (Daunay and Hazra 2012; Rotino et al. 2014; Devi et al. 2015; Plazas et al. 2016, 2020b), most of these crossing studies have been conducted for taxonomic purposes and preliminary breeding works, and the development of backcross generations was still not undertaken. In addition, the success rate of crossing eggplant with species from other genera or subgenera has been very low (Daunay et al. 1991).

As cultivated eggplant is self-compatible (Daunay et al. 1991), the frequent infertility characterizing the interspecific hybrids with its CWRs could be attributable to reduced affinity of the genomes involved in the cross, hampering a correct pairing of chromosomes at the meiosis and therefore inducing formation of irregular and sterile microspores and egg-cells. As a consequence, interspecific crosses with *Solanum* species yield eclectic results-ranging from abnormal hybrid plants, hybrids with normal phenotype but with sterile or partially fertile pollen/ovary, production F_1 fruits containing none, shriveled or non-germinating seeds, early fading embryos, until hybrids yielding viable progenies but with variable levels of fertility in the BC plants (Daunay and Hazra 2012).

Several attempts have been made to transfer key agronomic traits from wild and allied relatives into the eggplant background using different "unconventional breeding methods" (somatic hybridization, embryo rescue, and genetic transformation) (Daunay and Hazra 2012; Rotino et al. 2014). Successful fertility restoration by doubling the F_1 hybrid's ploidy level has been reported in the hybrid obtained by crossing eggplant and *S. aethiopicum* (Isshiki and Taura 2003) or *S. violaceum* (Isshiki and Kawajiri 2002). Relevant knowledge acquired on eggplant regeneration from different organs, tissues, cells, and protoplasts has resulted in the development of somatic hybrids (Rajam et al. 2008). This provided the opportunity to overcome sexual barriers between species to introgress alien genes for the genetic improvements of eggplant (Sihachakr et al. 1994; Collonnier et al. 2001a; Kashyap et al. 2003; Rajam and Kumar 2006). However, the obtained hybrids often resulted highly sterile, preventing their practical utilization (Gleddie et al. 1986; Guri and Sink 1988a; Sihachakr et al. 1988, 1989; Collonnier et al. 2003). Nonetheless, protoplast fusion between *S. melongena* with *S. aethiopicum* gr. *gilo* and gr. *aculeatum* was successfully employed to produce somatic hybrids which, after ploidy reduction by anther culture (Rizza et al. 2002) were successfully incorporated of into breeding programs to introgress useful traits from wild species into eggplant (Daunay et al. 1993; Rotino et al. 1998, 2001, 2005, 2014; Collonnier et al. 2001b). The so obtained advanced introgression breeding lines, already employed for the introgression of resistance traits to *Verticillium dahliae* and *Fusarium oxysporum* (Toppino et al. 2008a, b) into the genetic background of eggplant, could also be evaluated for their potential tolerance to abiotic stresses and exploited in breeding programs in particular for drought and salt resistance.

Amenable to in vitro culture, eggplant is potentially well-adapted to the exploitation of somaclonal variation. However, as genetic variability induced by hybridization and segregation is not yet been fully explored, somaclonal variation has less interest for this crop and so far, has not been much exploited (Collonnier et al. 2001a, b; Kantharajah and Golegaonkar 2004; Daunay 2008; Rajam et al. 2008; Rotino et al. 2014). Nevertheless, few somaclonal variants have been reported. For example, a salt-resistant line was obtained from cell culture in a medium containing 1% sodium chloride (Jain et al. 1988). Induction of lateral roots in cultures of eggplant grown in hormone-free liquid medium was used to demonstrate the role of polyamines in increasing resistance to abiotic stresses (Sharma et al. 1997; Kumar et al. 2006), as previously done for spermidine in stressed conditions (Sharma and Rajam 1995, 1997).

Eggplant anthers of six different varieties cultured under low temperature stress resulted able to induce some chilling tolerant anther calli in a genotype-dependent manner which could be of interest to generate improved varieties with better adaptability to stress for induction of chilling tolerance (Zhao et al. 2001).

Mutagenesis proved an effective strategy to induce variability into eggplant (Prakash et al. 2018). Eggplant was mutagenized with ethyl methane sulfonate (EMS) and 325 independent M2 families generated (Xiao et al. 2017a) which may be considered of interest to widen the genetic variability of eggplant could be employed for breeding purposes regarding adaptability to adverse environmental conditions.

4.8 Molecular Mapping, GWAS and Marker-Assisted Gene Introgression

Both inter- and intra-specific mapping populations and genetic maps were developed in eggplant. Overall, the interspecific maps benefit from an enhanced frequency of marker polymorphism, but their relevance to marker-assisted crop breeding is limited, although of extreme interest for the introgression of useful traits including tolerance to abiotic stresses from the allied and wild relatives of eggplant.

Among the interspecific linkage maps, the earliest constructed was based on the restriction fragment length polymorphism (RFLP) through genotyping of F_2 individuals bred from the interspecific cross *S. linneanum* x *S. melongena* (Doganlar et al. 2002). This map was improved by Wu et al. (2009) by adding COSII markers (Conserved Ortholog Set; Wu et al. 2006) previously mapped in the tomato genome. The improvement of the interspecific map previously established has been reported by Doganlar et al. (2014) by using more individuals and markers. With the aim to introgress *S. incanum* useful alleles involved in the biosynthesis of chlorogenic acid and the tolerance to drought into the genetic background of *S. melongena*, Gramazio et al. (2014) developed a interspecific (*S. melongena* × *S. incanum*) linkage map based on a 91 plants obtained from the first backcross (BC1) of the hybrid obtained with *S. incanum* with the recurrent eggplant accession *AN-S-26*.

The first true intraspecific map of eggplant was built up in 2001 by Nunome et al. (2001), subsequently improved in 2003 and 2009 (Nunome et al. 2009, 2003).

Barchi et al. (2010) developed two intraspecific mapping populations [a doubled haploid (DH) and an F_2] from the cross between the breeding lines '305E40' and '67/3'. As an extensive segregation distortion was observed in the DH population, only the sexual F_2 population was, later on, used for mapping purposes.

Subsequently, a great improvement in eggplant intraspecific map was performed by Fukuoka et al. (2012) using two intraspecific F_2 mapping populations, which were afterwards combined into one and used for QTL analysis of parthenocarpy in eggplant (Miyatake et al. 2012).

With the advent of next generation sequencing (NGS), single nucleotide polymorphism (SNP) markers have been developed based on the so-called genotyping by sequencing (GBS) approach, which included reduced representation sequencing (RRS) as well as whole genome resequencing (WGR) techniques.

Barchi et al. (2011) applied the protocol from Baird et al. (2008) to the genomic DNA of the F_2 segregating population obtained by crossing '305E40' and '67/3', used as female and male mapping parents, respectively. A new intraspecific map composed of SNPs and genotyped via Illumina GoldenGate© was developed (Barchi et al. 2012).

Hirakawa et al. (2014) built an intraspecific map for the anchoring of the draft genome sequence of the species and included both SNP and SSR markers, while more recently Salgon et al. (2017, 2018) developed two high-density intraspecific genetic maps using SNPs. Finally, Toppino et al. (2020) released a new ultra-dense linkage map from a F_6 recombinant inbred line (RIL) population, which was used for mapping quantitative trait loci (QTLs) related to seed vigour and plant anthocyanin content, as well as metabolic QTLs influencing fruit nutritional quality traits (Sulli et al. 2021).

To identify QTLs underlying key agronomic traits, biparental approaches as well as genome-wide association studies (GWAS) were conducted in this species, using the available linkage maps based on pre-NGS and/or NGS markers. So far, the interspecific and intraspecific mapping population developed, as well as the QTLs mapped, are mostly related with plant-fruit features and agronomical traits, fruit quality including biochemical composition and resistance to biotic stresses. Furthermore, these populations could be further exploited to map traits related to tolerance to abiotic stresses, particularly if detailed phenotyping highlight contrasting response between the involved parents.

Solanum linneanum may be a source of superior alleles for some agronomical traits but also for resistance to abiotic and biotic stresses. The mapping population developed from the interspecific cross *S. linneanum* x *S. melongena* (Doganlar et al. 2002; Doğanlar et al. 2014), was used to map, through composite interval mapping (CIM), 71 QTLs associated to morphological and biological traits, including leaf, flower, fruit characteristics, day to flowering and fruit set were identified (Frary et al. 2014).

Backcross progenies (BC_1) derived from the crossing between the *S. incanum* and eggplant have been used to construct an interspecific linkage map with the aim to develop introgression lines (Vilanova et al. 2010; Prohens et al. 2013, 2012) with improved tolerance to abiotic stresses. Recently, Gramazio et al. (2017) developed an introgression line population of *S. incanum* in a *S. melongena* background to map chromosomal regions associated with tolerance to drought.

The parents of ultra-dense linkage map recently generated by Toppino et al. (2020) based on RIL progenies, were found to be contrasting for NUE after being evaluated in response to low and high Nitrate (NO_3^-) supply, both in hydroponic and greenhouse pot experiments (Mauceri et al. 2020). The characterization of the RIL population under low N supply will undoubtedly allow the detection of QTLs/major genes related to NUE.

The feasibility of marker-assisted gene introgression for resistance or tolerance to abiotic stresses relies on the availability of markers closely linked to or in the gene/s of interest which help its introgression into the target genome. Marker-assisted breeding for other biotic and abiotic stresses is still underexplored. However, the availability of introgression lines with wild species that may harbour genes for resistance to stresses such as the one with *S. incanum* (Gramazio et al. 2017) may facilitate, through screening of the populations, the identification chromosomal regions responsible of the tolerance.

4.9 Biotechnologies Approaches

A promising way to improve eggplant tolerance to abiotic stresses could be the engineering of biosynthetic pathways associated with stress responses (Prabhavati et al. 2007a, 2007b). Plants grown in adverse conditions employ a wide range of mechanisms such as shifts in the plant physiology as well as the expression of genes in response to stress leading to the formation of a wide variety of low molecular weight metabolites, notably osmolytes and proteins able to cope with the stress condition (Rajam et al. 1998; Wei et al. 2019; Wang et al. 2020; Gong et al. 2021). Eggplant is well adapted to the application of genetic engineering as it responds well to *Agrobacterium*-mediated transformation with both cointegrate and binary vectors carrying *NPTII* (neomycin phosphotransferase II)*, CAT (catalase)*, and *GUS* (*β-glucuronidase)* reporter genes (Guri and Sink 1988a, b, c; Rotino and Gleddie 1990; Leone et al. 1993; Fári et al. 1995) as reporters. The bacterial mannitol-1-phosphodehydrogenase (mtlD) gene which is involved in the mannitol synthesis when expressed in eggplant caused tolerance to osmotic stress induced by salt, drought, and chilling (Prabhavathi et al. 2002). It also increased resistance against three fungal wilts caused by *F. oxysporum*, *V. dahliae* and *R. solani*. Mannitol levels could not be detected in the wild-type plants, but the presence of mannitol in the transgenics could be positively correlated with the disease resistance (Prabhavathi and Rajam 2007a). Polyamine-accumulating transgenic eggplants overexpressing the key biosynthetic gene *ADC* (arginine decarboxylase), display an increased tolerance level against several abiotic stresses such as salinity, drought, low and high temperature, and heavy-metal and also an improved resistance against *Fusarium oxysporium* (Prabhavathi and Rajam 2007b). Tolerance to moisture stress was accomplished by expression of the transcriptional activator (*DREBA1A*), controlling the expression of genes containing C-repeat/dehydration responsive element, under the control of the stress inducible promoter *rd29A* (Sagare and Mohanty 2012), which induced a significantly improved tolerance in the transformed plants to extended stress condition, while control plants were completely dried. In another experiment, the AtRD29A-mediated over-expression of the two *A. thaliana* genes C-repeat binding factor 3 (*AtCBF3*) and cold-regulated 15A (*AtCOR15A*) genes, both caused a significant increase in the proline content as well as of the levels of catalase and peroxidase activities compared with the wild type at 4 °C, while the relative electrical conductivity and the malondialdehyde diminished, thus promoting the cold adaptation process to protect eggplant plants from chilling stress (Wan et al. 2014). Transgenic eggplant lines expressing the isopentytranspherase (*IPT*) gene under the senescence-specific SAG12 promoter displayed a significantly delayed leaf senescence in the T1 generation and enhanced tolerance to drought and cold stress compared with wild type eggplants (Xiao et al. 2017b).

Vacuolar Na^+/H^+ antiporters provide the best mechanism for cope with excess salinity and restore ionic homeostasis in plants under salt stress. Transgenic eggplants expressing a wheat *TaNHX2* gene encoding a vacuolar NHX-type (Na^+/H^+) antiporter displayed under salinity stress (200 mM NaCl) stable leaf relative water and chlorophyll content, proline accumulation, improved photosynthetic efficiency, transpiration rate, and stomatal conductivity, reduced oxygen radical and hydrogen peroxide production associated with the significant increase in the activity of antioxidant enzyme with respect to the non-transformed plants (Yarra et al. 2019). Conventional breeding programs alone may be valuable, but the use of molecular methods to identify and introgress genes for stress-associated mechanisms may give an added advantage by surpassing incompatibility limitations of the former (Rotino et al. 2014; Toppino et al. 2021). Indeed, the use of biotechnologies combined with conventional breeding methods is the key to future improvement of eggplant. Biotechnological methods have been developed in eggplant for about a quarter of a century, and they are since then widely used in eggplant breeding processes. Thanks to the ability of the species to well respond in in vitro tissue culture, notably plant regeneration, several biotechnological techniques, including in vitro regeneration, protoplast culture/fusion and androgenesis, have facilitated the production of useful somatic hybrids and improved breeding lines. In addition, genetic engineering (transgenesis) is carried out since the 1980s and molecular mapping is developing since the end of the 1990s, gene editing has been recently employed and represents a powerful technique both for gene functional analysis and practical application (Collonnier et al. 2001a, b; Kashyap et al. 2003; Daunay 2008; Rotino et al. 2014; Barchi et al. 2019a, b; Maioli et al. 2020; Toppino et al. 2021). Moreover, the availability of a first draft sequence (Hirakawa et al. 2014) and, more recently, of an anchored and annotated sequence of eggplant (Barchi et al. 2019b, 2021), together with transcriptomic data of the reference genome provides power tools for eggplant breeding and the molecular mechanism research.

4.10 Eggplant Genome Sequencing and Functional Genomics

Thanks to the development of NGS technologies, the genome sequences of many plant species have been made available. In the Solanaceae family, high quality genome sequence were already released for *S. tuberosum* (The Potato Genome Sequencing Consortium 2011; Sharma et al. 2013; Hardigan et al. 2015), *Solanum lycopersicum* L. (The Tomato Genome Consortium, 2012; Hosmani et al. 2019) and *Capsicum annuum,* (Kim et al. 2014, 2017; Hulse-Kemp et al.2018).

In eggplant, the first unanchored draft of *S. melongena* genome sequence was released in 2014 (Hirakawa et al.). NGS data were obtained using 454 GS FLX Titanium (Roche Diagnostics, Basel, Switzerland) and HiSeq 2000 (Illumina, San Diego, CA) platforms. After assembly, a total of 33,873 super-scaffolds were obtained (total length:833.1 Mb; N50 length: 64,536 bases), covering about the 70% of its projected 1.2 Gb genome size genome. Finally, following annotation procedure, a total of 42,035 genes were predicted.

Recently an Italian Eggplant Genome Consortium (IEGC), which includes the University of Torino (Department of Agricultural, Forestry and Food Science (DISAFA), the CREA (Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria) Research Centre for Genomics and Bioinformatics of Montanaso Lombardo (LO), the University of Verona (Biotechnology Department), and the ENEA (Italian National Agency for New Technologies, Energy and Sustainable Economic Development) in Rome has obtained a high quality eggplant genome sequence of the eggplant inbred line '67/3' (Barchi et al. 2019a, b). The project was also funded by the Seed Companies Vilmorin & Cie, Rijk Zwaan and Enza Zaden Research and Development. Firstly, Illumina sequencing was carried out, resulting in an assembly length estimated to be about 1,163 Gb. The N50 and N90 lengths were 678.719 and 151.506 bp, respectively. Subsequently, by applying optical mapping technology to Illumina scaffolds, a final assembly, covering 1.22 Gb in 469 scaffolds with an N50 of 3.58 Mb was obtained. Finally, a RIL (F6) mapping population derived from the cross of the female parent between the DH line '305E40' and '67/3' was low coverage resequenced (~1X) and the genetic linkage map obtained used for anchoring the genome assembly to the 12 chromosomes. Following the annotation process, a total of 34,916 genes were predicted.

More recently, two additional high quality genome sequences were obtained in the species. In 2020, the eggplant inbred line 'HQ-1315' was sequenced by using a combination of Illumina, Nanopore, 10X genomics sequencing technologies and Hi-C technology (Wei et al. 2020). The size of the assembled genome sequence was ~1.17 Gb, with a contig N50 of 5.26 Mb and consisting of 36,582 protein-coding genes. Li et al. (2021) assembled the genome sequence of the Chinese accession 'GUIQIE-1', obtained by combining PacBio long reads and Hi-C sequencing data. The genome sequence spans 1155.8 Mb, with an N50 of 93.9 Mb. Overall, 35,018 high-confidence protein-coding genes were annotated based on multiple sources.

In parallel, Barchi et al. (2021) released a highly contiguous genome assembly of the eggplant '67/3' reference line, by Hi-C retrofitting of a previously released short read and optical mapping-based assembly. The sizes of the 12 chromosomes and the fraction of anchored genes in the improved assembly were comparable to those of a chromosome-level assembly obtained by Wei et al. (2020) and Li et al. (2021), with a scaffold N50 of 92.1 Mb. Furthermore, by resequencing 23 accessions of *S. melongena* representative of the worldwide phenotypic, geographic and genetic diversity of the species, and one each from the closely related species *S. insanum* and *S. incanum,* the first eggplant pan-genome and pan-plastome were constructed.

4.11 Transcriptomics, Genomic Selection, Gene Discovery, and Gene Prediction

4.11.1 Transcriptomes and Expression Analysis

The recent availability of a *S. melongena* draft genome sequence of cv. Nakate-Shinkuro (Hirakawa et al. 2014) and complete anchored and annotated genome sequences of eggplant lines 67/3 (Barchi et al. 2019a, b, 2021), HQ-1315 (Wei et al. 2020) and GUIQIE-1 (Li et al. 2021) provide an excellent opportunity for performing comparative analysis at the transcriptome level in eggplant. Great efforts have been focused at elucidating the molecular pathways and gene expression networks affected in eggplant and some wild relatives under different abiotic stresses through transcriptome analysis of sensitive/tolerant accessions under different environmental conditions. In addition, comparative transcriptomics was carried out to investigate molecular mechanisms underlying fruit-related traits as well as to develop molecular markers in eggplant.

By unravelling the gene networks associated with the different response to the abiotic stress, candidate genes involved have been identified, which could be subjected to silencing/overexpression for functional studies. Furthermore, the identification of superior allelic variants of candidate genes could represent a useful tool for the improvement of eggplant under stressed conditions.

Comparative transcriptome analysis was used to understand the salt-response mechanisms in leaves and roots of two eggplant accessions with different response to salt stress, highlighting genotype and organ-specific responses to stress. Furthermore, a K^+ transporter gene named *SmAKT1* was also identified, which contributed to higher tolerance to salinity in eggplant (Li et al. 2019).

The comparison of cultivated and wild (*S. sisymbriifolium*) eggplant species transcriptomes was performed with the purpose to characterize genes and selective patterns during eggplant domestication (Wei et al. 2019). A total of 19 genes that may have been positively selected during domestication were identified and divided into four groups, i.e., hormone response, development and response to disease and abiotic stress, oxidation–reduction pathway, and development. Overall, eight were potentially involved in abiotic stress tolerance or disease resistance, suggesting that environmental changes and biotic stresses were important selective pressures in eggplant domestication.

Regulation mechanisms may differ under different temperature treatments, in which heat shock proteins, antioxidant enzyme systems, detoxication, phytohormones, and heat stress transcription factors play vital roles (Zhang et al. 2020). In addition, high temperature significantly decreased the total anthocyanin content in peels. Indeed, at the early stage of peel coloring, high temperature limited the expressions of key genes in anthocyanin biosynthetic pathways by mean of transcription factors regulation, resulting in a significant decrease in total anthocyanin content, which might reduce the peel color in eggplant (Lv et al. 2019; Zhang et al. 2019).

Enzymes of the chalcone synthase (*CHS*) family showed a significant correlation with anthocyanin accumulation. Wu et al. (2020) identified seven *CHS* (*SmCHS1-7*) putative genes in a genome-wide analysis of eggplants (*S. melongena* L.). Under heat stress (38 °C for 3 h), the expression level of eight key genes similar to *SmCHS4* were found to be upregulated. Finally, the comparative analysis of putative CHS protein evolutionary relationships, cis-regulatory elements, and regulatory networks indicated that *SmCHS* gene family has a conserved gene structure and functional diversification, showing two or more expression patterns. Overall, these results may facilitate further research to understand the regulatory mechanism governing peel color in eggplants.

Gramazio et al. (2016) sequenced the transcriptomes of *S. incanum* and *S. aethiopicum*, providing a broad insight into gene sequences and allelic variation in the two species. Furthermore, by combining the newly generated information with the one available for *S. melongena* and *S. torvum* (available through NCBI database), intraspecific and interspecific polymorphisms were identified, and subsets of molecular markers were created for all species combinations, exploitable across all the eggplant gene pool for breeding programs.

Low temperature is one of the most threatening stresses affecting eggplant during its whole growth period, being responsible of growth and development inhibition as well as loss of yield. Yang et al. (2020a, b) compared the transcriptomics profiling of two eggplant varieties with differential low temperature tolerance. Results revealed significant differences in the plant hormone signal transduction and expression of some key transcription factor (including a *WRKY*) families between two varieties, thus providing a new insight into the molecular mechanisms involved in the response to low temperature in eggplant.

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNAs that play an essential role during plant development and stress responses. To elucidate the mechanisms of miRNA regulation in *S. aculeatissimum* under low-temperature stress, Yang et al. (2017) sequenced the transcriptome and small RNA and analyzed the correlation between mRNA and miRNA. Numerous conserved and novel miRNAs involved in the chilling response were identified, providing a theoretical basis for further studies of low temperature stress-related miRNAs and the regulation of cold-tolerance mechanisms of eggplant at the miRNA level.

Cadmium (Cd) is a heavy metal element harmful to human health. However, since the toxicity of Cd to plants is relatively low, Cd in the soil is taken up by crops and translocated to edible parts. *Solanum torvum* is a low Cd-accumulating plant and Cd content in the fruits of eggplant grafted onto *S. torvum* grown in Cd-polluted soils were found to be significantly lower than those of eggplant grafted onto other species (Arao et al. 2008). To elucidate the molecular mechanisms of the Cd acclimation process in *S. torvum* cv. Torubamubiga, a first study on transcriptional regulation in response to mild Cd treatment was carried out (Yamaguchi et al. 2010). Results showed that, beside the induction of heavy metal chaperone proteins, antioxidative and sulphur-assimilating enzymes were also upregulated. Furthermore, rapid repression of dehydration-related transcription factors and aquaporin isoforms suggests that dehydration stress is a potential constituent of Cd-induced biochemical impediments.

In contrast to *S. torvum*, *S. nigrum* is a cadmium (Cd) accumulator. To decipher the molecular mechanisms that are responsible for differential Cd accumulation in the two *Solanum* species, Xu et al. (2012) carried out a comparative transcriptome analysis. Results revealed a higher expression of the genes that encode several metal transporters as well as antioxidant-related genes, and several organic and amino acid biosynthesis/metabolism-related genes in Cd-treated *S. nigrum.* Overall, information obtained provided an insight into novel strategies that may be used for phytoremediation and food safety.

Recently, four genotypes of eggplant having contrasting NUE (Nitrogen-use efficiency) were subjected to genome-wide transcriptomic analysis (Mauceri et al. 2021). Aims of the study was to identify key genes related to the NUE pathway after short- and long-term low-N exposure, in both root and shoot. A key role in high-NUE genotypes was found for a *WRKY33,* responsible of triggering the expression of 21 genes including other transcription factors (TFs), many of which related to N-metabolism, able to improve both NUE components, i.e., NUpE (Nitrogen Uptake Efficiency and NUtE (Nitrogen Utilization Efficiency).

Finally, DNA methylation through the activity of cytosine-5-methyltransferases (C5-MTases) and DNA demethylases plays important roles in genome protection as well as in regulating gene expression during plant development and plant response to environmental stresses. The differential transcript profiling of C5-MTases and demethylases was assessed in leaves of the eggplant F_1 hybrid 'Nite Lady' subjected to salt and drought stresses suggesting a role for both class of genes in response to abiotic stresses in eggplant and providing a starting framework for supporting future epigenetic studies in the species (Moglia et al. 2019).

4.11.2 Genome Wide Identification—Families of Genes

It has been reported that many genes which play a crucial role in stress regulation and response or confer stress tolerance are mainly regulated by transcription factors (TF) (Lata and Prasad 2011). Indeed, transcription factors having an important role in stress signal perception and transduction processes could induce the expression of stress-responsive genes by recognizing and interacting with cis-acting elements in their promoter region, thereby the stress tolerance of plants is enhanced by activated stress signal cascade and whole downstream functional genes of this network.

Recently, a genome-wide identification from the eggplant genome database of the genes belonging to the DNA-binding with one finger (DOF) family was carried out (Wei et al. 2018). DOF is a group of plant-specific transcription factors that play important roles in plant growth, development, and response to biotic and abiotic stresses. Overall, 29 *SmeDof* members were identified, classified into nine subgroups. The phylogeny, gene structure, conserved motifs, and homologous genes *of SmeDof* genes were comprehensively investigated. Analysis of the expression patterns of

SmeDof genes in six different eggplant subspecies provided valuable information for understanding the mechanisms underlying the role of *SmeDof* TFs in abiotic stress response.

A genome-wide identification of the eggplant family of plant heat shock factors (*SmeHsfs*) was recently performed (Wang et al. 2020). As a consequence of high temperature stress, a large number of proteins in plant cells have to be denatured and inactivated. To avoid programmed cell death, heat shock factors (HSFs) and heat shock proteins (HSPs) are quickly induced to remove denatured proteins, thus enhancing the thermotolerance of plants. A total of 20 *SmeHsfs* genes were identified, and further classified into 14 subgroups on the basis of their structure. Real-time PCR expressions survey of the *SmeHsf* under various conditions revealed that *SmeHsfs* responded to four stresses, i.e., cold, heat, salinity and drought, suggesting that *SmeHsfs* play crucial roles in improving tolerance to various abiotic stresses. In particular, it was found that the expression pattern of *SmeHsfA6b* exhibited the most immediate response to all the various environmental stresses but drought. Overall, authors propose that the abiotic stress-responsive expression pattern analysis represents the starting point for deciphering the roles of *SmeHsfs* under environmental stresses and their regulatory mechanism.

More recently, Gong et al. (2021) identified a total of 24 *Hsf*-like genes and 117 *Hsp*-like genes in eggplant, using the interolog from Arabidopsis. The gene structure and motif composition of *Hsf* and *Hsp* genes were relatively conserved in each subfamily. Furthermore, by comparing RNA-seq and qRT-PCR data from a thermotolerant and a thermosensitive line, authors showed that the expressions of most eggplant *Hsf* and *Hsp* genes were increased upon exposure to heat stress, especially in thermotolerant one. The comprehensive analysis showed that different sets of *SmHsps* genes were involved downstream of particular *SmHsfs* genes, providing information exploitable for revealing the roles of *SmHsps* and *SmHsp* for thermotolerance in eggplant.

Another study reported on the identification of 58 genes annotated as eggplant WRKY all over the eggplant genome. These were further classified into three groups or seven subgroups in accordance with was previously reported in other plants (Yang et al. 2020b). Subsequently, two genotypes with different cold-tolerance were subjected to transcriptome sequencing, showing that some WRKY genes were differentially expressed in response to cold stress, suggesting that WRKY transcription factors could respond to cold stress and constituted a complicated network to regulate the cold tolerance in eggplant. Finally, two upregulated genes in both the accessions, i.e. *SmWRKY26* and *SmWRKY32* were silenced using virus induced gene silencing (VIGS), resulting in an increased sensitivity to cold stress.

Generally, C-repeat binding factors (CBFs) are crucial transcription factors involved in regulation of the response to abiotic stress, and especially in cold-response pathway, which might be applied to molecular breeding to improve cold tolerance of eggplant. Three eggplant C-repeat binding factor genes (*SmCBF1*, *SmCBF2* and *SmCBF3*) belonging to the A-1 group member of the CBF/DREB1 subfamily, were functionally characterized, and expression analysis revealed that these genes were all induced by cold stress, drought, high-salinity and abscisic acid (ABA) treatments

(Zhou et al. 2018). More recently, Huang et al. (2019) showed that *SmCBF* expression level is increased in eggplant fruits treated with eugenol (EUG) thus suggesting that EUG treatment had potential effect on alleviating chilling injury (CI) in eggplant fruit.

4.12 Future Prospects

Thanks to high-throughput genotyping technologies, many large-scale marker-trait association analysis, such as genome-wide association studies have been carried out, allowing to dissect the genetic architecture of plant traits of relevance for breeding. Furthermore, RNA-seq experiments allowed to capture the transcriptional changes in contrasting accessions for stress resistance/tolerance and to highlight the involvement of specific gene families in the direct response to environmental stresses which would deserve further functional characterization and employment for the genetic improvement of eggplant through biotechnological approaches.

Targeted modification of specific genes via genome editing is now used routinely to modify plant genomes (Osakabe et al. 2016). To date, several improvements to increase efficiency and specificity have been developed to generate heritable mutations in various plant species and also used to generate new mutant alleles of environmental stress response genes in plants (Osakabe et al. 2017, 2018). Even if for the moment no gene editing experiments are reported in eggplant for abiotic stress response, efforts spent in other species will be helpful in the further applications of genome editing to improve eggplant responses to adverse environmental conditions.

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Chapter 5
Genomic Designing for Abiotic Stress Resistant Brassica Vegetable Crops

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Abstract To meet the challenges of food and nutritional security, there is urgent need to increase production of quality vegetable crops. There are several environmental stresses which affect vegetable crop production and drastically reduce yield and quality of the crops. Abiotic stress triggers a series of changes in plants in respect to gene expression and cellular metabolism. Brassica vegetable crops play an important role in the diversification in cropping system and also in supplying micronutrients and other nutraceuticals. These cool-season crops are highly suffered during present climate change scenario. Thus, there is a need to search genotypes

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that can tolerate drought and excess water condition. An understanding of genetics and mechanism of stress tolerance will enable the development of suitable varieties for stress condition. Abiotic stress resistant Brassicas with improved agronomical traits can be achieved by combining the traditional methods with the modern biotechnological tools. Different studies on diverse plant groups also support the fact that miRNA entities play a major role during stress regulation and therefore, transgenic approach can be a promising tool for improving plant yield with tolerance to stresses. With the help of NGS based sequencing platforms has elucidated the gene regulatory network of abiotic stress resistance in Brassicaceae crops.

Keywords Brassica · Cole crops · Vegetables · Abiotic stress · Breeding · Genomics · Resistance

5.1 Introduction

5.1.1 Economic Importance of the Crop

Brassica vegetables are an important group of vegetable crops comprising cole crops like cauliflower, cabbage, broccoli, kohlrabi, Brussels sprout and kale and other crops like root vegetables (e.g. radish, turnip, swede), leaf vegetables (e.g. Chinese cabbage, vegetable mustard). Brassica vegetables belong to Brassicaceae, or mustard family (Maggioni et al. 2018; Murovecet al. 2018). These vegetables are among the most commonly cultivated vegetables in the world, and some of the most nutritious vegetables. The cultivated varieties of *Brassica oleracea* L. (Haynes et al. 2009) have 9 chromosomes and they are characterized by various morphological types. These have been differentiated under human selection which form the main economic part of the plants from a simple leafy type (leafy kale, var. *viridis* L.) into modified inflorescences in broccoli (var. *italica* L.) and cauliflower (var. *botrytis* L.), encircled leaves into 'heads' in cabbage (var. *capitata* L.), enlarged basal stem in kohlrabi (var. *gongylodes* L.), thickened stems in marrow-stem kale (var. m*edullosa* Thell.), proliferated heading buds in Brussels sprouts (var. *gemmifera* (DC.) Zenker), etc. The species *B. rapa* comprises morphotypes like Chinese cabbage and pakchoi (Chalhoub et al. 2014). The center of origin for cole crops is Mediterrianean region and from there it spread all over the Europe eventually. Among the Brassica vegetables, cauliflower and cabbage are the two most important crops grown while kohlrabi, broccoli and Brussels sprout are grown in certain areas, and yet to be as popular as the other two. Of the two, cabbage is more important covering nearly 2.47 mh with a production of 71.26 mt followed by cauliflower and broccoli covering 1.34 mha with a production of 25.23 mt. They are regarded as highly economically important crop in local and international trade as well.

5.1.2 Reduction in Yield and Quality Due to Abiotic Stresses

Currently climate change is creating a serious risk to global food security. Environmental constraints i.e. high and low temperature, drought, excessive moisture, salinity, alkalinity, atmospheric pollutants, and minerals in the soil affect Brassica vegetable production. Abiotic stress triggers a sequence of responses in plants which involve changes in gene expression and cellular metabolism (Santhiya et al. 2020). The stress response of the plants depends on the duration, severity frequency with which a stress is imposed the affected organs and tissues, developmental stage and genotype. Consequently, a combination of different conditions causes different plant responses to the same type of stress. At high and low temperatures, seed germination is drastically reduced. In arid and semi-arid region with poor irrigation, crop production is limited by low water table, high level of salinity and brackish water. These vegetable crops are highly susceptible to salinity. Atmospheric pollutants, especially ozone, inhibit the growth. Water scarcity and waterlogging are general problems in many parts of the world. Under drought conditions, there is drastic reduction in the yield and under excessive water condition, plant wilting is a normal feature. Under the adverse atmospheric condition Brassica vegetable production is highly restricted. Thus, there is a need to search genotypes that can tolerate various abiotic stresses. Developing a better understanding of genetics and mechanism of stress tolerance will enable the advancement of suitable varieties for stress conditions. It has been observed that millions of acres of crop loss occur every year due to multiple abiotic factors.

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

Brassica vegetables play a critical role in the diversification in cropping system and also in supplying quality food by meeting the nutritional requirement. For introducing several stress tolerance mechanisms for the improvement of crop performance, different apparently unrelated approaches are introduced into specific crop plants. These crops are generally considered to be cool-season crops and therefore, likely to suffer during present climate change scenario. They are highly reactive to extremes of drought, waterlogging, salinity, cold or other sources of abiotic stress (Abou-Hussein 2012). Many biotic and abiotic stresses are responsible for production and productivity reduction of Brassica vegetables. Therefore, the development of sustainable, high-yielding varieties with improved tolerance to various abiotic stresses is essential for meeting the global food demand (Patil et al. 2016). Generally, the Brassicacae family are considered as moderately tolerant to salinity. As compared to diploid species like *Brassica oleracea*, *Brassica nigra* and *Brassica rapa*, the amphidiploid species like *B. carinata, B. juncea* and *B. napus* have higher tolerance to salinity (Pavlovic et al. 2019). Among the Brassica vegetables, kale is considered as the most tolerant to salinity as compared to related varieties. Abiotic stresses have been reported to affect varying economic plant parts in Brassica vegetables. Heat stress mainly induces array of undesirable characteristics like riceyness, premature bolting, bracketing, irregular and rough head surface, reduced head or curd weight and diameter, leafiness, buttoning and undesirable coloring of head or curds. Breeding heat tolerant Brassica vegetables has potential and scope to expand the area and growing season of Brassica vegetables globally.

5.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

The emphasis must be given toward the production of high-yielding Brassica vegetables with good nutritional value that are environmentally sustainable and resistant or tolerant to extreme weather conditions. Breeding for tolerance against abiotic stresses by classical methods of selection and crossing is time consuming and often inefficient procedure. Various efforts have been taken up to resolve single stress under controlled conditions, but this approach is not always advisable because of difference in plant response under natural field condition where multiple factors and stresses prevails (Rizhsky et al. 2004). In the past decade, conventional breeding resulted in significant advances for wide ranging traits including biotic and abiotic stress tolerance, yield components and quality-related traits (Lin et al. 2014). Therefore, considering the genetic complexity and environmental interactions, application of more comprehensive and multidisciplinary approaches offers a better strategy to improve stress tolerance in modern crops (Sharma et al. 2011; Chaudhary et al. 2015; Chopperla et al. 2018). Improved salt and frost tolerance may be accomplished by the means of direct gene transfer or through DNA mutation. The new biological and molecular tools have unfolded new perspectives in stress biology and can be applied in these crops for developing biotic and abiotic stress tolerance crops.

5.2 Description on Different Abiotic Stresses

5.2.1 Root Characters Modification

Roots are the essential part for plant adaptation to various abiotic stress. Root system architecture (RSA) viz. root length, spread, number and length of lateral roots exhibits a great role in response to environmental changes. The RSA can be modified to have narrow root angle to the soil surface for lateral root development in the upper root regions where light penetration is the greatest. The profuse fine roots accommodating large surface area and root volume are essential for water extraction during soil moisture stress. In a previous study it was found that the specific root length of cauliflower was lower under drought stress conditions leading to a higher dry matter deposition in the fine root fraction (Kage et al. 2004a, b). Also, the vertical increment of rooting depth per degree day almost doubled under drought stress conditions (Kage et al. 2004a, b).

5.2.2 Heat Tolerance

Brassica vegetables are mainly gown in winter season and require cool climate for their growth and development. Most of the Brassica species grow normally at 15–22 °C temperature (Branham et al. 2017). The temperature above 35 °C and below 10 °C is detrimental to reproductive organs during different developmental stages of Brassica vegetables (Angadi et al. 2000). To expand production area, it is essential for Brassica vegetable to adapt high temperature. Variation of temperature during growth period imposes strong impact over the growth of cole crops. At high temperature, cabbage shows reduced disease and lower yield (Park et al. 2013). Stomatal conductance and fresh weight both are affected by air temperature. Kale is more sensitive to air temperature changes than cabbage (Rodriguez et al. 2015). In cauliflower and broccoli, curd or head development is regulated by temperature and require vernalization for transition from the vegetative to the generative phase to induce the formation of the edible part (Tan et al. 2000; Lin et al. 2015a, b; Matschegewski et al. 2015). The high ambient temperature delay vernalization, thus prolong vegetative phase and damage economic part. Moreover, excessive heat exposure during the vernalization sensitive phase often results in bracketing (Grevsen et al. 2003), whereas low temperature can induce premature flower bud development that causes riceyness (Grevsen et al. 2003), decrease quality and yield, such as non-uniform head size, bracketing within heads, uneven head surface, decreased head weight and diameter, and undesirable colors (Farnham and Bjorkman 2011a, b; Branham et al. 2017).

The fluctuation in temperature also affects the growth and development in different crops like riceyness in cauliflower (Zhao et al. 2020). The heat tolerance trait in the interspecific somatic and sexual hybrids between cabbage (*B. oleraceae* var. *capitata*) x Chinese cabbage *(B. campestris.* var. *peknensis*), and between Chinese kale (*B. oleracae* var. *alboglabra*) x Chinese cabbage was intermediate between of the parents (Huang et al. 2003).

5.2.3 Cold Tolerance

Cold/freezing is a critical environmental stress that is disadvantageous to plant growth and development, and can limit crop productivity too (Xin and Browse 2000; Ahmed et al. 2015). Brassica that is seeded in northern climates where winter is severe, may be affected by genetic variation for other cold-regulated traits, such as freezing tolerance, vernalization responsive flowering time and leaf characteristics (Kole et al.

2002). Cold acclimation is crucial for freeze tolerance of membrane, as demonstrated by cold-acclimated cabbage and spinach that synthesize proteins responsible for protecting thylakoid membranes against freezing damage. Low-temperature stress also influences specialized metabolism in Brassica plants. Low temperature stress induces accumulation of specific flavonol glycosides and hydroxycinnamic acid derivatives in *Brassica oleracea* var. *sabellica* (Neugart et al. 2016). Besides phenolic compounds, low-temperature regimes may be linked with higher carotenoid accumulation (Mageney et al. 2016). Glucosinolates play a vital role in the abiotic stress response (Martínez-Ballesta et al. 2013).

5.2.4 Drought Tolerance

The drought can occur at any stage throughout a plants life cycle. In Brassica vegetable crops, economic part initiation and development stage are the most susceptible stage. Drought stress in cauliflower reduced seed germination, shoot and root length and biomass, stomatal conductance, transpiration, curd growth and dry matter (Hnilickova et al. 2004; Kage et al. 2004a, b; Hadi et al. 2014). In Chinese kale, both water deficit led to reduced leaf area, fresh and dry weight and leaf number, with drought leading to darker leaves and closed stomata (Issarakraisila et al. 2007).

5.2.5 Flooding and Submergence Tolerance

Oxygen starvation, that is a outcome of the relatively slow diffusion of gases in water by plant roots is caused by flooding (Takeshiand and Julia 2004). Water logging resulted in rapid leaf chlorosis in cauliflower (Shih et al. 2013). Most of the cauliflower cultivars cannot tolerate flooding. Thus, heavy rainfall poses a high risk for cauliflower production (Lin et al. 2015a, b). Hsu and Wu (2019) found the variety Fuyodori had higher flooding tolerance and the line 228 had lower flooding tolerance. In Chinese kale, water logging led to reduced leaf area, fresh and dry weight and leaf number, with drought leading to darker leaves and closed stomata (Issarakraisila et al. 2007). Short periods of water logging stress led to cultivar differences in cauliflower and broccoli in characters like heading percentage, yield per unit area, root dry weight, protein expression and growth scores (Higashio et al. 2012; Chen et al. 2014; Lin et al. 2015a, b).

5.2.6 Other Abiotic Stresses

Salt stress has become an ever-increasing threat to Brassica vegetable production. It is a major factor limiting the crop productivity. Increased salinization of arable lands is expected to have a devastating global effect, resulting in 30% land losses within the next 25 years and up to 50% by the year 2050 (Zheng et al. 2004). Increasing concentration of soluble salts can be harmful to the plant. High salinity has been found to reduce shoot and root length, dry weight and fresh weight in cauliflower, broccoli, kale and cabbage (Shannon et al. 2000; Giuffrida et al. 2013; Wahid et al. 2014).

5.3 Genetic Resources of Resistance Genes and Gene Pool

The Brassicaceae family constitutes about 4060 species under more than 372 genera (Bayer et al. 2018; Singh et al. 2019b). The appraisal of crop genetic diversity analysis is essential for implementing efficient conservation and breeding practices for the development of climate resilient varieties (El-Esawi et al. 2016). The most appropriate strategy to enhance the resilience in modern varieties of crop plants is exploiting the wild allies in breeding programs. The Brassicaceae family comprises numerous species of research interests like model plant Arabidopsis, *B. napus, B. rapa, B. juncea, B. oleracea, Eruca sativa, Sinapsis alba* and *B. carinata* (Quezada-Martinez et al. 2021). The U triangle exhibiting cytogenetic relationship among Brassicas generated by Nagaharu U (U 1935) exhibits both diploid (*B. rapa, B. oleracea, B. nigra*) and amphidiploid (*B. napus, B. juncea, B. carinata*) species. This large and complex gene pool constitutes the "section Brassica" of the "genus *Brassica*," the taxonomy of which remains controversial. The gene pool of Brassica is given in Fig. 5.1. Helm (1963) proposed a scheme as to how the various taxa of the cabbage group developed. He suggested that the oldest type var. *ramose* (thousand-head kale) which gradually gave rise to var. *gemmifera* (Brussels sprouts) probably in Belgium,

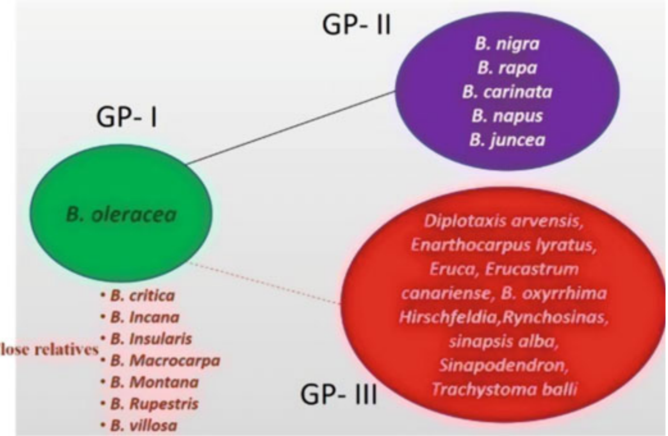

Fig. 5.1 The gene pool of Brassica

were developed from wild cabbage var. *sylvestris*. The wild cabbage also developed into a stock var. *acephala* from which var. *costata* (Portuguese kale) was evolved in Portugal. Cabbage (var. *capitata*) and savoy cabbage (var. *sabauda*) are both derived from this Portuguese kale, the latter one in Italy. Similarly, the kales and collards (vars. *acephala, sabellica, selensia and palmifolia*) were steadily developed from the same stock which also produce the forage crop, marrow-stem kale (var. *medullosa*). Helm (1963) also suggested that Pliny's Pompeian cabbage could be an ancestor of this type. Kohlrabi (var. *gongylodes*) is derived from the same form or from marrow-stem kale. Sprouting broccoli (var. *italica*), primarily developed from wild *sylvestris*, bring about cauliflower (var. *botrytis*). Yarnell (1956) considered that *B. cretica*, *B. insularis* and *B. rupestris* may have contributed to this development. These species are inter-fertile with *B. oleracea* and, therefore, have been classified in one cytodeme by Harberd (1972). The amphidiploid species have been reported to exhibit higher salinity tolerance as compared to diploid species of U triangle (Ashraf et al. 2001). Among the Brassica vegetables, Chinese cabbage has the ability to accumulate and tolerate high concentration of heavy metals. Despite of the numerous problems linked with the utilization of wild allies for the genetic improvement of crop plants, this method presents a vital potential for the improvement of Brassica crops, to make them resilient to global climate change.

The availability of novel genetic diversity is essential to extend the range of resistance or tolerance to numerous abiotic stresses like drought, cold, heat, salinity and water logging. Soil salinity, one of the major abiotic stresses affecting Brassica vegetables and to characterize the markers linked to tolerance in Brassica crops, Pavlovic et al. (2019) studied the comparative analysis between cabbage, Chinese cabbage and kale for salinity tolerance. Their results reported kale as most tolerant to salinity and Chinese cabbage is susceptible to salinity stress. Mutation has also played a significant role in enhancing genetic variability in crop plants. In this context, the *N*-nitroso-*N*-ethylurea (NEU) and *N*-Nitroso-*N*-methyl-urea (NMU) induced cauliflower mutants have been reported to tolerate low temperature and salinity conditions (Fuller et al. 2006).

5.4 Glimpses on Classical Genetics and Traditional Breeding

5.4.1 Classical Mapping Efforts

Almost all the Brassica vegetables are adopted to cool season, they are highly sensitive for heat stress and require specific average temperature for optimal growth and development. Increase in average annual temperature and heat shocks as a result of global warming is threat to ideal production; hence it is the need of the hour to develop heat tolerant cultivars in case of Brassica vegetables. The major issues that the crops have included are susceptibility to several biotic and abiotic stresses and seed composition traits. Improving agronomic performance of the crop shall ensure higher productivity and production, increased consumption of Brassica vegetables and increase in realized economic benefits. Traditionally, plant breeders have used crossing approaches coupled with meticulous selection methods to select better performing genotypes. Conventional plant breeding approaches led to development of many crop varieties. Breeding efforts for Brassica vegetables, like any other crop, are directed towards improving stable yield potential of the crop. Classical genetics and traditional breeding approaches have been used to develop tolerance for drought, water logging stress, salt tolerance and for other abiotic stresses. For past few decades the improvement of Brassica vegetables for abiotic stresses has been targeted and much progress have been achieved. To serve this purpose, numerous tools have been employed such as applications of different omics approaches for deciphering genes linked with abiotic stress tolerance (Raza et al. 2021). However, the major developments in this arena are the identification of stress responsive genes, marker development, quantitative trait locus (QTL) mapping, transcriptomics analysis, and elucidation of cellular and physiological mechanisms in response to various abiotic stresses.

5.4.2 Breeding Objectives

Advanced phenotyping-based breeding approaches are pre-requisite and being adopted systematically by developing bi-parental, backcross or multi-parent intercross populations using drought tolerant exotic and/or indigenous parental lines and wider-adaptable high yielding varieties. After two cycles of selection, intensive selection of candidate genotypes for multiple drought tolerance related traits is practiced in advance generations using three-tier selection scheme followed by multi-traits indexing.

5.4.3 Classical Breeding Achievements

The classical approach uses sexual cycle to recombine DNA through crossing over for genetic variation. Induced mutations are also included in classical breeding approaches. In any of these approaches, emphasis is on the selection of individual plants and their progenies (Hall 1992; Wang et al. 2004). Therefore, it is imperative to know about suitable selection criteria, which differ for each species and stress. The natural selection in heterogeneous populations of crop plants should increase the frequency of genes for exorbitant temperature (Grover et al. 2003; De Vos et al. 2005). If stress tolerance genes are not available in cultivated types, these can be transferred from the wild relatives/species that are the rich source of stress tolerance genes. In traditional breeding approaches, heat tolerant varieties were developed such as Punjab Kunwari, Early Kunwari, Pusa Early Synthetic, Pusa Kartik Sankar, Pusa Meghna, Kashi Kunwari, and Pusa Ashwini in cauliflower, and Pusa Agethi in cabbage. However, traditional varieties are susceptible to multiple stresses at different locations. This difficulty can be overcome by the transfer of genes for abiotic stresses from the wild varieties and their co-specific subspecies. If the tolerance level of the agronomically improved cultivars is not sufficient, the collections of the crop species may be screened for sources of desired variability. This variability may be used in the hybridization of the on-going program (Saulter et al. 1990). Some other traits like constitutive proline accumulation seems to be determined by oligogenes (Singh 2003).

5.4.4 Limitations of Traditional Breeding and Rationale for Molecular Breeding

The traditional plant breeding methods and selection procedures are time consuming for generation and characterization of breeding material for target traits. Improvement of Brassica vegetable crops for stress tolerance can be attained through the direct insertion of small number(s) of precisely selected alleles. A number of promising strategies including conventional and advanced tools have been employed to develop stress-tolerant genotypes. However, for these approaches to become successful the performance of stress-tolerant germplasm must perform as well as elite lines under normal non stressed growth conditions and it has to outperform elite lines when grown in a stressful environment. To overcome these stresses only few scientific approaches are available, one by making use of certain tolerant varieties/rootstocks or by the use of pesticides etc. But there are very few tolerant varieties are available to overcome abiotic stresses and rootstock is one of the viable option. In this context the cauliflower's mutated population was generated to evaluate the resistance against abiotic stresses. It was demonstrated that mutant lines of cauliflower can be used as a source for breeding against salt and frost tolerance (Fuller et al. 2006).

Classical breeding, that uses interspecific or intergenetic hybridization achieved little success for the development of stress tolerant crops. Lack of efficient selection criteria in classical breeding method results in low genetic variability of crops under stressed environment. Therefore, stress tolerant crops open a new door to overcome these difficulties. For improving the genetic constituent of crop plant, traditional breeding methods like selection, hybridization, polyploidy and mutation are generally used (Das and Das 2019). Over the past three decades the molecular markers has been extensively utilized for analyzing the genetic diversity in crop plants including Brassica vegetables (El-Esawi et al. 2016; Singh et al. 2021a, b). The microsatellite or simple sequence repeat (SSR) markers have been the marker of choice for many years to study the genetic relationships and genetic divergence. Under the current era of climate change both genomics and phenomics will be instrumental in the analysis of genetic variation among crop wild relatives and landrace germplasm (Redden 2013).

5.5 Brief on Diversity Analysis

The appraisal of crop genetic diversity analysis is essential for implementing efficient conservation and breeding practices for the development of climate resilient varieties (El-Esawi et al. 2016). The geographical origin of primitive domesticated leafy kale, from which all the other cultivated forms were probably derived according to literary and linguistic findings, remains unclear. The availability of novel genetic diversity is essential to extend the range of resistance or tolerance to numerous abiotic stresses like drought, cold, heat, salinity and water logging. Soil salinity, one of the major abiotic stresses affecting Brassica vegetables and to characterize the tolerant Brassica crops, Pavlovic et al. (2019) studied the comparative analysis of salinity tolerance among cabbage, Chinese cabbage and kale. Their results reported Kale as most tolerant to salinity and Chinese cabbage as susceptible to salinity stress.

5.6 Association Mapping Studies

Genome wide association studies (GWAS) has been proved instrumental for characterizing the genetic constitution of the traits and determining the candidate genes governing those target traits. The genetic architecture of abiotic stress resistance in different crop plants has been revealed by employing GWAS tools (Bac-Molenaar et al. 2016). For the GWAS across the crop plants, single nucleotide polymorphisms (SNPs) are the marker of choice and for the generation of high-throughput SNP primers, specific locus amplified fragment sequencing (SLAF-seq) is an excellent method (Sun et al. 2013). The novel loci and candidate gene resistant to drought stress have been identified in other Brassica crops like rapeseed (Shahzad et al. 2021) and Chinese cabbage (Liu et al. 2014). The limited work has been done with respect to unraveling genetic architecture of abiotic stress resistance in Brassica vegetables. In the Chinese cabbage, the drought and salinity stress impose drastic impact on growth and yield of these crops globally. The ability of plant to tolerate such type of stresses depends on the plant's capability to express an array of genes regulated by specific transcription factors (TFs). The different families of transcription factors governing such genes are NAC, bZIP, AP2/ERF, WRKY and MYB (Alves et al. 2013; Liu et al. 2014). The NAC genes are specific plant transcription factors and plethora of NAC genes have been observed in the model plant Arabidopsis (Kawaura et al. 2008). These NAC genes perform multiple biological functions in the plant. The completion of high-quality genome sequence of *Brassica rapa* provides a remarkable opportunity for genome wide studies of these genes. Based on the genome wide analysis studies Liu et al. (2014) classified the *BrNAC* gene family in Chinese cabbage into eight groups. Under the different abiotic stress conditions (drought and cold) numerous members of *BrNAC* gene family were upregulated in Chinese cabbage. GWAS facilitates cloning of QTLs for different abiotic stress resistance traits. GWAS have been employed in cauliflower (*Brassica oleracea* var. botrytis) for mapping of curd related traits (Thorwarth et al. 2018) and for identification of flowering related QTLs (Matschegewski et al. 2015). The cauliflower is highly thermo-sensitive crop, therefore genetic assessment of temperature-dependent curd regulation in cauliflower by GWAS and gene expression analysis is highly important. In this context, GWA mapping revealed significant loci linked with floral transition which is useful for breeding cultivars to perform under different temperature regimes (Matschegewski et al. 2015).

5.7 Molecular Mapping of Resistance Genes and QTLs

5.7.1 Evolution of Marker Types

The molecular markers have shifted over the years from tedious hybridization-based restriction fragment length polymorphisms (RFLP) to more robust and versatile polymerase chain reaction (PCR)-based random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and SSR marker types. However, molecular markers like SSR, SNP, diversity array technology (DArT) and expressed sequence tag (EST) are more consistent and widely used in the recent times. Availability of numerous molecular markers and QTL mapping techniques have paved the way for good understanding of genetic basis of vital agriculturally significant traits such as yield, nutritional quality and resistance to various abiotic and biotic stresses (Xue et al. 2010; Ali et al. 2013). Construction of linkage map by assigning molecular markers is an important step for analyzing the genome of a particular crop for future molecular breeding and selection for desired traits. Therefore, linkage maps have been used for QTL identification, marker assisted breeding, dissection of quantitative traits, map based cloning strategies, genome organization and comparative genomics of predominant species (Paterson et al. 2000; Dekkers and Hospital 2002). The use of molecular markers in genomic studies of *Brassica* species was started with the development of first RFLP linkage map for *B. oleracea* (Slocum et al. 1990) followed by *B. napus* (Landry et al. 1991) and *B. rapa* (Song et al. 1991). RAPD and RFLP have been extensively used in *Brassica* species for phylogenetic and genetic mapping studies (Williams et al. 1990). RFLP, RAPD, SSR and AFLP have been used in Chinese cabbage for construction of linkage maps (Choi et al. 2007; Kim et al. 2006; Soengas et al. 2007) and in *Brassica oleracea* PCR-based markers are used for the same in the recent past (Cheung et al. 1997; Sebastian et al. 2000). Earlier SSR and AFLP marker were more commonly used for the construction of linkage maps and those linkage maps with limited molecular markers and less density which restricted the QTL identification and related studies (Zhang et al. 2011). Presently, next generation sequencing (NGS) techniques have made it possible to develop large number of SSR and SNP markers. Hence the developed SSR and SNP markers will empower the selective breeding by the means of genomic selection by facilitating the fine mapping of QTLs and improvement in identification and utilization of genes influencing important traits (Gao et al. 2012; Zhao et al. 2012). Throughout genome distribution of SSR and SNPs makes these markers more preferred for genetic and genomic studies (Barchi et al. 2011; Xu et al. 2011; Bus et al. 2012) therefore, these are ideal for association and fine mapping of genes to identify potential alleles affecting agronomic and horticultural traits (Huang et al. 2010; Blanca et al. 2012). Large number of SNP (1,228,979) and SSR (20,836) markers have been characterized in non-heading Chinese cabbage (Song et al. 2015). Huang et al. (2017) mapped 3985 SNP markers over 10 linkage groups in Chinese cabbage whereas, Yang et al. (2020) has identified a total of 827,720 SNP loci with 257 newly developed SNP markers. Xu et al. (2019) reported 70,475 insertion/deletion (InDel) markers, 706,269 SNPs, and 71,942 SSRs in three cabbage genotypes.

Breeding Brassica vegetable crops tolerant to various abiotic stresses have caught the breeder's attention globally with the goal of adaptation of improved varieties under changing climatic situations. Inheritance of majority of the traits associated with the tolerance to abiotic stresses is polygenic and complex in nature. Therefore, genetic markers developed to identify the QTLs associated with stress tolerance traits could be used to accelerate the genetic gains in these quantitative traits through marker assisted selection and gene pyramiding (Branham and Faranham 2019). Statistical tools or software like MAPMAKER/QTL, MapQTL, MQTL, PLABQTL, QTL Cartographer, QGene, QTLMapper, QTL Network are used to identify the chromosomal positions (loci) controlling quantitative/polygenic traits (Andleeb et al. 2020). These identified chromosomal positions of the genomic region are called as QTLs. QTLs controlling the trait of interest could be a single gene or cluster of genes. These are characterized as consecutive and adaptive QTLs based on the stability of QTL effects across the different environmental conditions, where consecutive QTLs are detected across all the environments whereas an adaptive one increases their expression with the level of environmental factor or only spotted in specific environmental conditions (Collins et al. 2008). Via integration of QTL and eco-physiological models Yin et al. (2003) proposed the use of phenology model in plant breeding.

5.7.2 Mapping Populations

Mapping population is usually derived from F_1 plants that are created as a result of genetic union between the two phenotypic diverse parents for a target trait. The simplest type of mapping population is F_2 population which is the result of one meiosis consisting recombined hereditary material and the level of polymorphism can be detected using molecular markers or at phenotypic level. Three different F_2 populations were exploited to identify the 86 QTLs controlling eight different curd related traits in *B. oleracea.* An F_2 population of 196 individuals derived from salt tolerant (2205) and salt sensitive parent (1423) were used for construction of QTL mapping in *B. napus* (Lang et al. 2017). Asghari et al. (2008) used 350 SSR and 250 RAPD markers to access the parental polymorphism among F_2 and F2:3 (F_2 derived F_3 population) population for the linkage map construction and QTL identification, associated with the winter survival and related traits in *B. napus*. Two commercial breeding lines BLM25 (superior curd quality and horticultural characters along with late curding time) and BLM 29 (early curd forming kale derived broccoli line); were crossed to develop F_2 population to identify QTLs associated with curd induction and curd quality in *B. oleracea* var. *italica* (Lin 2018). $F_{2:3}$ population derived from a cross of (VI-158 × BNC) was also used for QTLanalysis of glucosinolates in broccoli (Brown et al. 2015).

Mapping population of F_2 individuals was used for mapping of lobed-leaf gene linked with the salt tolerance by using QTL technology to locate the QTLs related to lobbed leaf formation in *B. napus* (Zhang et al. 2018). One of the major drawbacks of using F_2 population is that these are temporary and are not immortal therefore difficult to maintain for long and cannot be used for comparative studies. Lack of availability of mapping population limits the Brassica vegetables crop improvement furthermore, presence of self-incompatibility makes it difficult to maintain these populations (Sebastian et al. 2002; Pink et al. 2008). Different marker systems, variable germplasm and variability in linkage group nomenclature has been limiting the attempts of amalgamation of existing genetic maps (Hu et al. 1998) therefore, restricting the information integration among the research groups.

Doubled haploid (DH) and recombinant inbred line (RIL) populations are immortal populations and are more often used for construction of high-density genetic maps and detection of QTLs. DH is the ideal population for genetic analysis of quantitative traits of polygenic inheritance due to their genotypic reproducibility across the different environments and provide robust data for QTL analysis. F_1-derived DH lines progressed from a cross between annual cauliflower and Brussels sprout were used to detect the QTLs for various developmental and morphological characters (Sebastian et al. 2002). A DH population derived from a cross between broccoli and Chinese kale was developed for quantitative genetic mapping approach in order to analyze the low temperature stress (de Jonge 2016). A DH population was constructed containing 130 lines from F_1 plants via microspore culture to detect the QTLs related to head transverse diameter and head shape index in cabbage (Zhang et al. 2016). Su et al. (2015) also developed DH population to develop a high-density genetic map in cabbage. A DH mapping population, derived through microscope culture of F_1 from a cross between two advanced homozygous diverse inbred cabbage lines, was used to construct a high-density genetic map in cabbage (Wang et al. 2012). DH16–2 (purple sepals) and DH28–4 (green sepals), lines were crossed to get DH lines resultant from an F_1 hybrid in order to construct a high-density genetic map and identification of QTLs controlling purple flower head in broccoli (Yu et al. 2019a). Others also used DH population for construction of high-density genetic map in broccoli for QTL identification associated with various horticultural and biochemical traits (Stanwell et al. 2019; Voorrips et al. 1997; Yu et al. 2019b; Li et al. 2021). An RIL population consisted 150 ($F_{2:7}$) lines derived from a cross between 06 and 247 and He102 followed by single-seed descent method in Chinese cabbage for the construction of high-resolution mapping for identification of QTLs controlling main floral stalk length (Liu et al. 2019). One hundred and sixty, RILs developed in *B. rapa* and 155DH lines were developed in *B. oleracea* from an F_1 cross between two

Table 5.1 Transgenic *Brassica* species for abiotic stress tolerance

Transgenic plant	Type of abiotic stress	Transgene	References
Brassica campestris	Salt stress	LEA4-1	Park et al. (2005)
Brassica chinensis	High temperature and salt stress	AgcodA	Wang et al. (2010)
Brassica oleracea var. *botrytis*	Salt stress	APX, SOD	Metwali et al. (2012)
Brassica oleracea var. *botrytis*	Enhanced proline production and frost resistance	CBF/DREB1 and COR15	Hadi et al. (2011)
Brassica rapa	Salt stress	BrGI	Kim (2016)

DH lines in broccoli for construction of immortal population and genetic linkage map in both the species (Iniguez-Luy et al. 2009).

In the recent past attempts have been made to detect the QTLs responsible for regulating flowering and heat tolerance in order to reduce the area and season dependency for their production. QTL detected in several Brassica vegetables in response to several abiotic stresses are listed in Table 5.1. Curd initiation and development is highly temperature dependent in *Brassica oleracea* var. *botrytis* due to vernalization requirement which often results in uneven maturity, delay or sometimes no curd development under high temperature conditions, therefore Rosen et al. integrated the genetic analyses with phenology modeling to hasten the breeding stratagem towards wide-adaptation cauliflower. The study was carried out with DH lines for detection of QTLs where, 10QTLs for leaf appearance rate, five for the slope and two for the intercept of linear temperature-response functions were detected. Genome-wide association and gene expression analysis were performed to examine the temperature-dependent curd induction in cauliflower (Matschegewski et al. 2015) where 18 identified QTLs were located on chromosomes O1, O2, O3, O4, O6, O8, and O9 for curding time under different temperature regimes, with many of the QTLs localized within the region of candidate flowering time genes. Flowering locus *BoFLC2*, is associated with the flowering time in cauliflower (Okazaki et al. 2007; Ridge et al. 2015); therefore Lin et al. (2019a, b) investigated the effect of different temperature on apex development and gene expression of *BoFLC2* in tropical type of cauliflowers including early (H-37: heat tolerant) and mid to late (H-80) maturity types. The expression of *BoFLC2* in 'H-37' was significantly down regulated as compared to H-80 after curd initiation and advance growth, however the expression of *BoFLC2* disappeared at higher temperature in both types of cauliflower suggesting that temperature stress hinders flowering time in cauliflower at higher temperature of above 30 °C.

5.7.3 QTL Mapping Software

For past few decades, researchers are emphasizing on QTL studies for detecting the specific QTLs of various traits in Brassicas. But the major disadvantage of QTL studies includes low accuracy and false positive results. However, the adaptation of different QTL mapping methods proved to be a good way for increasing the accuracy of QTL mapping. Various effective statistical analysis like single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM), multiple interval mapping (MIM), and Bayesian interval mapping (BIM) are also used for getting a precise map position. To serve this purpose, numerous QTL mapping softwares like Mapmaker/QTL, QTL Cartographer, MapQTL, PLABQTL, PGRI, MapManager, QTLMAPPER, QGene, QTLSTA, Ici Mapping, and QTL network are available. Nevertheless, complex inheritance of unstable QTLs has become a grueling issue in this context.

5.8 Marker-Assisted Breeding for Resistance Traits

The difficulties of selection process during classical plant breeding approach can be overcome employing improved competence and accuracy of selection (Collards et al. 2005). For combining various desirable characteristics into a distinct genotype, higher numbers of conventional breeding series are needed. In Brassica crops, genome research with the application of marker assisted program came to light in the late 1980s when the first RFLP linkage map for *B. oleracea* was published (Slocum et al. 1990). Contrarily, marker assisted selection (MAS) is a more accurate method that uses less series for pyramiding desirable traits into a specific genotype and thus resulted in less inaccuracy (Babu et al. 2004). Therefore, MAS decreases the time and resources while enhancing the yield of the crops. Most of the time, farmers ignore the low yield genotypes of land races, wild varieties or progenitors although, these genotypes confer good resistance or tolerance against abiotic stress. However, MAS has shown a path for the crop improvement by utilizing markers to screen stress tolerance genotypes and thus regarded as one of the pivotal technique for crop improvement (Nezhadahmadi et al. 2013; Tuberosa 2012). On the basis of the long terminal repeat and BoTPPI-2 sequences, to identify HTHH tolerant genotypes, Song et al. (2020) developed a molecular marker and validated it in F_2 individuals, inbred lines, and cultivars from diverse sources. This marker confers only upto 80% accuracy in cabbage genotypes.

5.9 Map-Based Cloning of Resistance Genes

Map-based cloning is the most widely used method to clone resistance genes that usually follow gene-for-gene interactions. It is also called positional cloning as it identifies the genetic basis of mutant phenotype by simply looking for linkage to earlier reported markers (Jander et al. 2002) or it finds the underlying cause of genetic variations. Therefore, more focused research is needed in molecular mapping and tagging of disease resistance genes. No prior information about specific genes is needed in this and therefore it is one of the most preferred methods to clone disease resistance genes in the plants. In recent years, many resistance genes (R gene) have been identified in cole crops and successfully applied for their improvement against various diseases. Target genes or traits in map-based cloning are those genes that are majorly responsible for providing resistance to disease. As the whole genome sequences and information about several markers of many plant and crop species are available, map-based cloning has become easier. Map-based cloning of resistance genes uses the approach to decrease the distance between the gene of interest (here resistance gene) and the analyzed markers. For mapping of resistance genes, we need to find markers that are closely associated with our gene of interest. In plants, like Arabidopsis where the entire genome is sequenced, mapping becomes comparatively easier. In crops, where genomic information is not well available, one needs to develop simple sequence length polymorphism (SSLP) markers de novo, followed by identification of sequence segments having SSLP, development of PCR primers, and its testing. Techniques like AFLP are highly reproducible and therefore do not even require information about prior sequences and can be used directly in any plant system for mapping and cloning purposes (Vos et al. 1995). AFLP markers based on the genome-wide mapping are used when information about the position of a gene is available. Shimizu et al. (2015) identified the candidate gene "*FocBo1*" conferring yellow wilt resistance in *B. oleracea.* The *FocBo1* locus was fine-mapped by exploiting F_2 plants derived from resistant cabbage and susceptible broccoli DH lines within 1.00 cM between BoInd2 and BoInd 11 markers. Analysis of BAC (bacterial artificial chromosome) and cosmid sequences identified an orthologous gene of *Bra012688* that confers yellows resistance in Chinese cabbage. A linkage and segregation analysis of markers with resistance genes assist in the identification of resistance or tolerance genes in plants. YAC (yeast artificial chromosome), BAC and TAC (transformation-competent artificial chromosome) clones are important tools used for developing the fine structure of map by making clone libraries. These clone libraries should contain several clones that usually overlap each other. Then each of these clones is ordered, which depends upon sequence-tagged sites (STS). For overlapping of two clones (YAC, BAC, or TAC), the PCR products should amplify from any two clones. As the clones are from the original DNA source, these STS do not need to be polymorphic. Availability of a large-insert genomic DNA library is important for the positional cloning of genes. YAC cloning system can clone large DNA fragments of up to a size of 1000 kb (Burke et al. 1987). However, YAC itself is very unstable in yeast host strains and is difficult to purify from the host. Another large insert clone system has been developed to overcome these difficulties of YAC. BAC, TAC, and PAC (P1 derived artificial chromosome) cloning system is extensively used to make genomic libraries in plants, due to several advantages such as large insert size (<300 kb), high cloning efficiency, and stability of foreign DNA (Ming et al. 2001). Two approaches practiced for map-based positional cloning of genes are chromosome landing and chromosome walking. In chromosome walking, first, the DNA marker that is linked to the target resistance gene is identified, and then ‘walking steps’ are taken to get to the genes by a series of overlapping clones. The markers that are closely linked to the target genes are used to screen the genomic library to identify the positive clones. Positive clones are a means to isolate inserts’ ends. To screen the library for more overlapping clones, these insert ends are used as the probe. The entire process is repeated multiple times to walk across the chromosome and reach the target gene. Chromosome walking is however not favorable in crop plants due to large genomic size, presence of highly repetitive sequences, and is time-consuming (Tanksley et al. 1995). Chromosome landing depends on identifying the molecular markers that are tightly linked or co-segregate with the target gene. There should be less physical distance between these markers and the target gene. To find whether the cloned resistance gene is transformed or not a complementation test is done. The DNA fragments cloned are digested by different restriction enzymes and inserted into binary vectors for complementation tests. These constructs are inserted into the host using either the *Agrobacterium*-mediation method for transformation or other methods like electroporation, particle bombardment, etc. The selection of transformants is based on the presence or absence of resistance genes. For expression analysis, RT-qPCR is done which suggests us the levels of expression of mRNA.

5.10 Genomics-Aided Breeding for Resistance Traits

The genus “*Brassica*” includes one of the economically important plant groups, however, their cultivation and production are threatened by the emerging biotic and abiotic stresses. Therefore, the gene of interest and the mechanisms involved in tolerance/resistance should be recognized (Poveda et al. 2020). Traditional methods to control these stresses are costly and have limited effects. Therefore, the development of genomics, transcriptomics, and biological techniques enables us to discover resistance (R) genes rapidly (Lv et al. 2020). Recently, sequencing projects on several crops have further enhanced our understanding of their genomic structure, and evolution, function, and utilization for crop improvement. The exploitation of these techniques for increasing resistance against biotic and abiotic factors will be the priority in Brassica crop improvement (Augustine et al. 2014). Functional genomics is used for understanding the functions of genes and proteins by utilizing genome-wide approaches. It provides the information about gene expression, protein function and translation, protein–DNA, protein–RNA, and protein–protein interactions. Apart from functional genomics, structural genomics is also widely used as this is a branch that deals with the exploration of protein structures on the genomic level. It aims to characterize the structure of all proteins within a specific target set, such as every protein encoded by a specific genome including areas that were previously beyond our technological capabilities (Chandonia et al. 2004; Bunnik and Roch, 2013; Saad et al.2021). Jung et al. (2017) identified 17 calcineurin B-like (CBL) genes from the Chinese cabbage database and *Br135K* microarray datasets employed in calcium signaling to regulate responses to environmental stimuli. Using microarray analysis, the responsiveness and the differential expression of *BrCBL* genes to cold, drought, and salt stresses were confirmed, which can be used for the selection of candidate genes for functional characterization. Kayum et al. (2017) have identified 32 PDI (protein disulfide isomerase) genes in Chinese cabbage, out of which 24 PDI genes were upregulated under salt and drought stress. Nozawa et al. (2017) analyzed a heat-activated retrotransposon, named ONSEN, in *Brassica* vegetables. A high copy number of OLEs in *B. oleracea* has been found to instigate transpositional silencing using epigenetic regulation such as DNA methylation and would be advantageous to the understanding of adaptations to environmental stress.

Brassica napus L. comprises 37 drought resistant cultivars related genes, 35 drought sensitive cultivar related genes and 97 cultivar non-specific genes that are grouped into different functional categories. By the help of network analysis, it is established that, these 169 differentially expressed genes (DEGs) of different chromosomes plays a vital role in response to drought tolerance in *Brassica napus.* Similar expression profiles of *Hsf* genes from *B. napus* and their analogous genes from *B. oleracea* and *B. rapa* revealed the existence of high functional similarity between these genes. This study helps in understanding the *Hsf* genes and their role in improving stress tolerance in *B. oleracea.* Shan et al. (2019) described the *BobHLHs* gene and found its phylogenetic relationships, expression patterns, and response to chilling stress in *B. oleracea.* Out of 234 genes, 21 showed organ/tissue-specific expression, six were downregulated in cold stress. The role of bHLH family members and BobHLH genes against chilling stress in cabbage was unfolded through this study. Dolatabadian et al. (2020) identified 1749 resistance gene analogs (RGAs), having 996 as core and 753 as variable, 368 being absent from the reference genome. Nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes are mostly laid in clusters. One hundred six RGA candidates were identified that are associated with blackleg resistance QTLs. This study throws better light for the understanding of resistance genes that are responsible for improved disease resistance and genomic based improvement of the cultivar. MAS is successfully applied for improving disease resistance (Mehraj et al. 2020). Arias et al. (2021) identified candidate genes and gene expression patterns that differ among morphotypes (3958DEGs) through transcriptome-wide differences that regulate the vegetative to reproductive transition in kale. Genes involved in morphology, nutrition, and defenses were differentially expressed. Using Bayesian phylogenetic approach, Kawakuboa et al. (2021) analyzed 579 whole genome sequences and upto 713 partial sequences of *Turnip mosaic potyvirus* (TuMV). They reported that from a particular center of origin, the TuMV isolates of the Asian-*Brassica*/*Raphanus* (BR) and basal-BR groups and world-Brassica3 (B3) subgroup was spread to the rest of Eurasia. Therefore, this study represented a detailed picture of the major transmission routes of the pathogen

(Kawakuboa et al. 2021). Wang et al. (2020a, b) compared the designed reference genome (*Brassica rapa*, IVFCAASv1) and revealed the mechanism of some differential gene expression levels after low temp treatment in non-heading Chinese cabbage through transcriptome and small RNA combined sequencing analysis. Out of 1860 new genes identified, 1613 were found to be functionally annotated. Further, from 13 DEGs, seven were found to be upregulated and six were downregulated, and the common differentials miRNAs were found to be eight.

5.11 Recent Concepts and Strategies Developed

5.11.1 Gene Editing

Various abiotic stresses are managed in nearly 20 crop species by employing clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) method of gene editing (Ricroch et al. 2017). Feng et al. (2013) demonstrated CRISPR/Cas9 based target genome editing for the first time in Arabidopsis. However, modern biotechnological approaches for breeding and research still do not use these gene editing tools rapidly in Brassica species. In recent times a few studies have been published where genome editing was performed using *A. tumefaciens* (Yang et al. 2017a, b).

Although CRISPR/Cas9 system is used vigorously as a potential genome editing tool for plant related research (Xiong et al. 2015; Schaeffer and Nakata 2016), but, in Brassica, only a few successful genome editing studies have been reported till date (Lawrenson et al. 2015; Braatz et al. 2017; Yang et al. 2017a, b; Sun et al. 2018). One of the examples was from the DH genotype AG DH1012 (a broccoli-like Brassica) which is a cross of the *Brassica oleracea* var. *alboglabra* (A12DHd) × *B. oleracea* var. *italica* (Green Duke GDDH33) where *GA4* gene was knocked out using CRISPR/Cas9 (Lawrenson et al. 2015). The genome sequence of cabbage (*Brassica oleracea* var. *capitata*) is now available and by implementing the CRISPR/Cas9 system, genetic as well as agronomical trait improvement of cabbage can easily be done (MA et al. 2019). Thus, CRISPR/Cas9 system can transfer the abiotic stress tolerance genes (T genes) very effectively replacing the sensitivity genes (S genes) in *Brassica* (Zafar et al. 2020).

Despite of being a very new technique, numerous researchers have published reports in high impacted journals about the implementation of CRISPR/Cas9 system for successful genome editing in a wide number of plant species. In *Arabidopsis thaliana* transgenic plants displayed retarded growth were developed by targeting *BR11*, *JAZ1*, *GAI* gene (Sprague et al. 1943). In *Brassica oleracea* transgenic plants displayed dwarf phenotype were developed after being subjected to targeted mutagenesis by targeting *BolC.GA4.a* gene (Chilcoat et al. 2017). Lawrenson et al. (2019) reported a successful gene editing protocol using *Agrobacterium* mediated tool that delivers Cas9 and dual sgRNAs in *Brassica oleracea.* It is proved to transform many stable and inheritable mutations at the targeted locus very efficiently.

5.11.2 Nanotechnology

Nanotechnology is another new tool that involve novel properties of nanomaterials for crop improvement program (Carmen et al. 2003; Kole et al. 2013). Nanoparticles mediated agronomical quality development of plants are often a concentration dependent process and that can up-regulate the activities of antioxidant enzymes like, SOD, CAT and POD (Laware et al. 2014). Reduced osmotic potential and Na + ion toxicity can occur due to the salinity stress on the plants. Pure unmodified alumina nanoparticles (13 nm) can reduce root elongation in cabbage that results in retarded growth of cabbage plant (*Brassica oleracea*) (Yang et al. 2005).

5.12 Genetic Engineering for Resistance Traits

To cope up with diverse environmental conditions, plants have developed various mechanisms involving several physiological and molecular pathways to respond and withstand the adverse conditions of nature. The present section highlights the key regulatory role of miRNAs, which play key roles in differential gene expression in *Brassica* spp. during stresses and various developmental processes. Different studies on diverse plant groups also support the fact that these miRNA entities play a major role during stress regulation; therefore, transgenic approach can be a promising tool for characterization of various miRNAs which can be deployed for improving plant yield, nutritional quality, value addition, and tolerance against various types of stresses in Brassica.

Genetic engineering mostly exploits the genes linked with regulatory and signalling pathways to confer the abiotic stress resistance in plants. Various stress inducible genes are identified that can be used for the improvement of stress resistance if induced in susceptible plants through genetic transformation. Last two decades proved to be an era of biotechnology that uncovered numerous functionally active genes that are associated with various metabolic processes like signal transduction and particle transport (Gill et al. 2013). Over the past few years, researchers have identified numerous genes that encodes for resistance genes against abiotic stress and transformed these genes into sensitive genotypes for acquiring improved abiotic stress tolerant varieties that are summarized in Table 5.1. Strategies employed for incorporating numerous abiotic stress tolerant genes into *Brassica* species are discussed briefly.

Over expression of *BoAOS* gene, obtained from cabbage can increase the endogenous jasmonic acid level in transgenic Arabidopsis plants and thus exhibit drought tolerance to Arabidopsis. *BoAOS* may be a suitable candidate gene to produce transgenic plants with tolerance to drought stress. The non-coding RNAs and multiple plant stress responsive mechanisms have been postulated to fight with plethora of abiotic stresses. In response to abiotic stress, the cellular metabolism of plants generates reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ion (O^{2-}), and hydroxyl radicals (OH^-) which culminates to cell death (Verma et al. 2019). To scavenge such ROS species, antioxidative defense mechanisms have been developed by different organisms. For instance, the effect of superoxide dismutase (*SOD)* gene family in response to stress incidence have been well studied in the Brassicaceae family including model plant Arabidopsis (Verma et al. 2019).

The constitutive and alternative RNA splicing events regulate the plant response to various abiotic and biotic stresses. Myriad of other key plant proteins like serine/arginine-rich (SR) proteins have been demonstrated to modulate the abiotic stress response by regulating the alternate splicing of essential plant signaling genes (Muthusamy et al. 2020). For instance, the enhanced expression of drought stress responsive *SR45a* gene has been reported in *Brassica rapa* (Muthusamy et al. 2020). The alternate splicing (AS) events in some of the genes of model plant Arabidopsis and key *Brassica* speciesl ike*B. rapa, B. oleracea* and *B. napus* are evolutionary conserved. In *Brassica rapa* 25 SR genes responsive to heat, cold and oxidative stress have been identified (Darracq and Adams 2013).

5.13 Role of Bioinformatics as a Tool for Abiotic Stress Tolerance

Bioinformatics plays a key role for combating various challenges that leads to the improvement of plant genetic constituent. Genomes of a number of *Brassica* species, that includes; *B. oleracea* cultivars 02–1212, TO1000DH13, and HDEM14; *Brassica oleracea* L. var. *botrytis* cultivar C-8 have been reviewed over past few decades (Liu et al. 2014; Parkin et al. 2014; Belser et al. 2018; Sun et al. 2019). However, by developing appropriate bioinformatics tools and applying complete genomic knowledge, abiotic stress resistant genes can be incorporated into sensitive genotypes by using CRISPR/Cas9 genome editing tool. Bioinformatics plays a crucial role in predicting the specific target sites for CRISPR/Cas9 genome editing (Doudna et al. 2014; Osakabe et al. 2016). One such example is the web-based tools E-CRISP (Heigwer et al. 2014). *Arabidopsis thaliana* Stress Responsive Gene Database (ASRGD) is a reference gene database that one can be used for exploring various genetic data of Arabidopsis (Borkotoky et al. 2013). Another public database, DroughtDB (Alter et al. 2015) provides detailed information about computed ortholog genes of nine model and crop plants. It includes manually curated genes involved in the drought stress response.

5.14 Brief Account on Social, Political and Regulatory Issues

The above technological advancement has evolved as a highly specific and efficient tool for crop improvement with the potential to rapidly generate useful novel phenotypes. This leads to the emergence of new plant breeding technologies such as to allow the investigation of gene functions and inducing variations for crop improvement. A question arises as to how genetically edited plants with desired traits will be received by the public and regulated within legislation on genetically modified organism (GMO). According to a recent survey comparing scientist and citizen views on a range of science, engineering and technology issues (Funk et al. 2015), the most pronounced difference obtained from the study was found on the question addressing the safety of consuming genetically engineered crops; whereby 37% of the public at large responded that genetically modified (GM) foods are generally safe to eat, whereas 88% of scientists interviewed recognized GM foods as generally safe (Wolt et al. 2016). Until the legal opinion is released, the legal status of living organisms as well as products deriving from New plant breeding techniques (NPBT) approache is unclear (Sprink et al. 2016). In the United State, the coordinated framework for regulation of biotechnology (CFRB) determined that it is the final product of genetic engineering that potentially poses a risk to human health and the environment, not the process by which the product is made.

5.15 Future Perspectives

Abiotic stresses (cold, drought, heat, salinity) are significantly impeding the growth of agriculture and sustainable development goals. As discussed above, the technological advancement provides a simple, robust, and potential system for performing functional genomics research in Brassica. For further development of Brassica research, steady and persistent efforts for standardization of CRISPR/Cas9 genome editing, transgenic approaches and marker assisted breeding system are need to be carried on. Thus, numerous Brassica varieties that are resilient to emerging pests and abiotic stresses can be produced. The technology also believed to have high potential for improving plant-breeding approaches to incorporate improved and beneficial traits in currently cultivated Brassica vegetables. Further crop improvement can be achieved by developing genome maps and identifying molecular markers for genes of interest across various different crop species. Agrochemical usage can also be minimized by developing stress resistant Brassica crops. Thus, high yielding crops can be obtained with minimized production cost. It can be concluded that, abiotic stress resistant Brassicas with improved agronomical traits can be achieved by combining the traditional methods with the modern biotechnological tools. These improved varieties are proved to be high yielding and environmental friendly as well. The challenge of ensuring global food security regardless of increasing population and the stresses intervened through changing climate is driving the demand for exploiting state-of-the-art genomics tools to enhance agricultural productivity. With the help of NGS based sequencing platforms and analysis of differentially expressed genes through RNA-seq or QTL-seq has elucidated the gene regulatory network of abiotic stress resistance in Brassicaceae crops. Much of the progress has been made in the oilseed *Brassica* crops which provide basis for extending the abiotic stress resistance in *Brassica* vegetables.

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Chapter 6
Genomic Designing for Abiotic Stress Tolerance in Cucurbits

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Abstract The Cucurbitaceae provide food and sustenance almost the entire world over, in economically advanced areas as well as resource-poor areas, and encompass over two dozen crop plant species. Worldwide, the mostly widely grown and consumed cucurbits are watermelons, melons, cucumbers, squash, and pumpkins. As with all crops, yield quantity and quality of cucurbits is determined by genetics, the environment, and management, and interactions among these three variables. Like other crop plants, the growth, yield, and quality of cucurbits is adversely affected by abiotic stresses. This overview is devoted to the possibility of developing cucurbits more tolerant of abiotic stresses by manipulating their genomes. Descriptive background information is provided on cucurbit plants and their distribution in the wild and under cultivation, breeding achievements, and the abiotic stresses affecting cucurbits. The presentation then discusses the limitations of traditional cucurbit breeding for stress tolerance, diversity analysis of cucurbits, association mapping in cucurbits, gene identification through genome-wide analysis, genome-wide association studies for identifying quantitative trait loci, prospects and limitations for marker-assisted breeding for stress tolerance in cucurbits, and the rather limited progress and insights gained so far from research on cucurbit genomics. Much more research in genomic designing for abiotic stress tolerance of cucurbits needs to be conducted, by screening of germplasm collections for abiotic stress tolerance and by discovering the target genes and their locations within the genome.

Keywords Cucurbits · -Omics · Molecular markers · Abiotic stress · Genome

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6.1 Introduction

1. Economic importance of cucurbit crops

The gourd family, Cucurbitaceae, is a monophyletic family that originated in southern Asia during the Cretaceous Period (Renner and Schaefer 2017). The Cucurbitaceae consist of approximately 1000 species assigned to 96 genera that are naturally distributed mostly in the tropics and sub-tropics. Typically, cucurbit plants are large, fast-growing, procumbent or climbing herbaceous, tendril-bearing, self-compatible monoecious vines bearing yellow or white radially symmetrical nectar-producing flowers foraged by bees, and the pistillate flowers have inferior ovaries containing numerous ovules.

More than two dozen cucurbit species have been brought into cultivation for food production (Table 6.1). Wehner et al. (2020) list over 50 cucurbit species that are at least sparingly cultivated or of which wild plants are gathered for food. Generally, the fruits of wild cucurbits are small, several centimeters in diameter, round, and green, and the flesh is often extremely bitter due to alkaloid compounds known as cucurbitacins. A fruit can contain as many as several hundred seeds, which, at maturity, contain two cotyledons each but no endosperm. The seeds are not bitter and are oval and flat, around 10 mm in length. Domestication of some of the Cucurbitaceae is ancient, predating that of some major grain crops. There is evidence that pumpkins were first cultivated over 10,000 years ago.

The cultivated cucurbits differ from their wild counterparts most obviously in their larger and fewer vegetative and reproductive parts (Schaffer and Paris 2016). They have larger leaves, thicker stems, fewer branches, and larger and fewer fruits and seeds. In some cultivated forms, fruit shape is not round but distinctly elongate. Cultivated cucurbits also differ sharply from their wild relatives by having fruits that are more palatable, being nonbitter and less coarsely fibrous, but higher in starch, sugar, and carotenoid contents and can exhibit an array of colors. A wealth of variability for fruit size, shape, and color occurs in the Cucurbitaceae.

Cultivation of cucurbits spans six continents, with over 80% of worldwide cucurbit production in Asia (McCreight 2017). Most production is conducted in the open field, but there is an ever-increasing proportion of protected cultivation. Almost always, cucurbits are cultivated for the consumption of their fruits, though in some cases, the flowers, seeds, seed oil, and young shoots are consumed. Generally, cucurbits are best adapted for production in warm regions but are often shipped in large quantities over long distances to lucrative marketing outlets in temperate and cool regions.

The most widely grown cucurbit crops worldwide are watermelon (*Citrullus* Schrad. spp.), cucumber and melon (*Cucumis* L. spp.), and pumpkin and squash (*Cucurbita* L. spp.). FAO (2020) statistics place these five cucurbit crops among the leaders of all vegetable crops in area planted and quantity of production, with the monetary value of each running into billions of dollars annually. Each of these five is produced in over 100 countries and their production per capita has been increasing steadily worldwide for years (McCreight 2017). Of these, watermelon leads in production and land area, with over 100 million tonnes produced on over

Table 6.1 Cultivated species of the Cucurbitaceae (after Maynard and Paris 2008; Chomicki et al. 2020)

Species	English name(s)	Continent of primary distribution in cultivation
Benincasa fistulosa (Stocks) H. Schaef. and S. S. Renner	Tinda	Asia
Benincasa hispida (Thunb.) Cogn.	Wax gourd, winter watermelon, ash gourd, Chinese watermelon	Asia
Citrullus amarus Schrad.	Citron watermelon	Africa
Citrullus colocynthis (L.) Schrad.	Colocynth	Africa
Citrullus lanatus (Thunb.) Matsum. & Nakai	Watermelon, dessert watermelon	Worldwide
Citrullus mucosospermus (Fursa) Fursa	Egusi watermelon	Africa
Cucumis anguria L.	West India gherkin	Africa
Cucumis melo L.	Melon	Worldwide
Cucumis metuliferus E. Mey. ex Naud.	Horned melon, jelly melon, kiwano	Africa
Cucumis sativus L.	Cucumber	Worldwide
Cucurbita argyrosperma C. Huber	Cushaw, silverseed gourd	North America
Cucurbita ficifolia Bouché	Fig-leaf gourd	South America
Cucurbita maxima Duchesne	Pumpkin, winter squash	Worldwide
Cucurbita moschata Duchesne	Tropical pumpkin, winter squash	Worldwide
Cucurbita pepo L.	Summer squash, pumpkin, winter squash	Worldwide
Cyclanthera pedata (L.) Schrad.	Stuffing cucumber, lady's slipper	South America
Lagenaria siceraria (Mol.) Standl.	Bottle gourd	Ubiquitous
Luffa acutangula (L.) Roxb.	Ridge gourd	Asia
Luffa aegyptiaca Mill.	Sponge gourd	Asia
Momordica charantia L.	Bitter gourd, balsam pear	Asia
Sechium edule (Jacq.) Sw.	Chayote	North America
Sicana odorifera (Vell.) Naud.	Casabanana	South America
Telfairia occidentalis Hook. f.	Oysternut, fluted pumpkin	Africa
Trichosanthes cucumerina L.	Snake gourd	Asia
Trichosanthes dioica Roxb.	Pointed gourd	Asia

3 million ha in 2018 (FAO 2020); cucumbers are next, with 75 million tonnes on almost 2 million ha, followed by melons with 27 million tonnes on slightly over 1 million ha and pumpkins, squash, and gourds, collectively, with 28 million tonnes on slightly over 2 million ha. Except for melons, production of these cucurbits at least doubled over the period 1994–2018. The other cultivated cucurbits are distributed regionally or locally, and can be found in gardens or on small farms in the warmer parts of the world.

Cucurbit fruits are popular because of the sweetness, texture, and flavour of watermelons and melons, and the diverse culinary adaptation of pumpkins, squash and cucumbers (Schaffer and Paris 2016). They are also increasingly recognized as an important part of a balanced diet in many countries, providing vitamins, minerals, anti-oxidants and dietary fiber. Watermelons contain considerable amounts of the anti-oxidant pigments lycopene and beta-carotene, ascorbic acid (vitamin C), potassium, and citrulline, a non-protein amino acid having several health benefits (Perkins-Veazie et al. 2012). Melons contain very high levels of vitamin C, and orange-flesh melons also have high amounts of carotenoids (Lester 2019). Pumpkins and winter squash are rich in anti-oxidant pigments, mostly lutein and the vitamin A precursors, alpha- and beta-carotene (Paris 1994). Immature cucurbit fruits, including summer squash and cucumber, contain lower amounts of vitamins, minerals, anti-oxidants, and dietary fiber. Nonetheless, summer squash contain fair amounts of each, considerably more than cucumbers (Gebhardt and Thomas 2002; Wehner et al. 2020). The seeds of some cucurbits are rich in oil and protein, with pumpkin production for extraction of its seed oil being an established, large industry in Austria and other European countries (Teppner 2000). Seed oils of various cucurbit taxa are used as salad dressing or for frying and cooking (Mariod et al. 2009). Cucurbit fruits can also contain additional anti-oxidants, such as flavonoids, but the total anti-oxidant activity of the various cucurbit fruits remains to be studied. Cucurbit fruits also contain numerous bioactive compounds, including vitamin E, biotin, and folic acid.

Many of the cultivated cucurbits, such as cucumbers and summer squash, are grown for the culinary use of their immature fruits, no more than one week after anthesis (Schaffer and Paris 2016). Others, including watermelons, melons, pumpkins, and winter squash, are grown for their mature fruits. Quality traits desired in mature cucurbit fruits in some cases are the same as those desired in immature cucurbit fruits but differ in other cases. Some characteristics, such as sweetness and flesh color, are fully expressed in mature fruits but hardly at all in young fruits. Other characteristics, such as size of the seed cavity, can be important in determining the palatability of immature fruits but are less important in mature fruits. A few, most notably pumpkins and squash (*Cucurbita* spp.) and melons (*Cucumis melo* L.), have been grown for the dual usage of their immature fruits as well as their mature fruits, or for the use of immature fruits in some regions but for the use of mature fruits in other regions, and as a consequence the species of pumpkins, squash and melons possess a great deal of intraspecific variation in fruit shape and other characteristics. Deviation from the ancestral spherical fruit shape results in proportionally more fruit exocarp and mesocarp (fruit flesh), and less of the seedy and stringy endocarp and seed cavity, hence summer squash and cucumber have been selected from time immemorial for elongate fruit shape. The large differences in fruit shape of cucurbit-crop cultivars are determined from early stages of ovary development until and immediately after anthesis (Sinnott and Durham 1929; Sinnott 1937; Paris 1986; Périn et al. 2002). Fruit size is also under genetic control, but environmental conditions and intraplant competition for photoassimilates play strong roles in determining fruit size.

The five cosmopolitan cucurbits are large, annual, tendril-bearing herbaceous plants, spreading as much as 1–4 m in each direction, with a life cycle of 3–4 months (Schaffer and Paris 2016). Cucumber, pumpkin and squash plants are monoecious, most watermelons are monoecious and some andromonoecious, and melons are mostly andromonoecious and some monoecious. The plants are self-compatible, and inbreeding depression is not common. Heterosis, though, is common and most commercial seed is hybrid. The flowers are yellow, borne in leaf axils and are relatively large, 2–3 cm in diameter in cucumber, melon, and watermelon, and orange-yellow to orange, 12–20 cm in pumpkin and squash. Male (staminate) flowers are differentiated initially and female (pistillate) or perfect (hermaphroditic) flowers later in plant development, but the female flowers grow faster. The flowers open in the early morning and are functional for only several hours after opening, withering thereafter. Pollinations are conducted by bees. Fruit set can become obvious as soon as the day after pollination.

The five cosmopolitan cucurbit crops are derived mainly from seasonally arid parts of four continents (Paris 2016a, b). Watermelons are native to Africa, whilst cucumbers and melons hail from Asia and pumpkins and squash are originally from North and South America (Chomicki et al. 2020). To ancient peoples, the cucurbits were a source of sustenance and water but, initially, their exploitation must have been difficult. Wild cucurbit fruits are small and, usually, extremely bitter. Quite likely, the first step in their exploitation was the nurturing of the occasional cucurbit plants that were found to produce non-bitter fruits, non-bitterness in cucurbit fruits being conferred by a single recessive gene (Navot et al. 1990; Paris and Brown 2005; Dogimont 2010–2011; Weng and Wehner 2016–2017). An additional step was the favouring of plants that produced larger fruits. Fruit size is a quantitatively inherited trait and the effect of each successive genic increment for larger fruits was likely exponential rather than additive (Sinnott 1937). Subsequently, fruits with increased palatability were selected, a process that is ongoing to the present day. Modern cultivated cucurbits are quite distant in appearance and taste from wild cucurbits.

There are seven species of watermelons, all of which have 11 pairs of chromosomes ($2n = 2x = 22$) (Chomicki and Renner 2015). One, *Citrullus lanatus* (Thunb.) Matsum. & Nakai, the dessert watermelon, is by far the most widely grown, familiar and greatly appreciated for its wateriness and sweetness (Levi et al. 2017). The weight of the multidisciplinary evidence, which encompasses archaeology, literature, linguistics, iconography and, lately, genomics, suggests that the beloved dessert watermelon is native to northeastern Africa (Paris 2015; Renner et al. 2017, 2019; Wu et al. 2019). It has been cultivated in the Nile Valley for several thousand years and has undergone major genetic bottlenecks in its evolution under domestication, resulting in a narrow genetic base (Levi et al. 2004).

Cucumbers, *Cucumis sativus* L., have only 7 pairs of chromosomes ($2n = 2x = 14$). They are native to the Indian subcontinent (Sebastian et al. 2010) and the word "gherkin" has been traced etymologically back to that region (Paris et al. 2011). As compared with the other cosmopolitan cucurbit crops, cucumbers seem to have been relatively recently domesticated, for 3000 to perhaps 4000 years (Paris and Janick 2010–2011; Paris et al. 2012a). Cucumbers are phenotypically less diverse than the other leading cucurbits, with little deviation in fruit diameter.

Melons, *Cucumis melo* L., have 12 pairs of chromosomes ($2n = 2x = 24$). They also are native to Asia (Sebastian et al. 2010). They have been in cultivation for quite some time and early on they diffused to Egypt, where long-fruited melons have been cultivated for over 4000 years (Janick et al. 2007; Paris 2012). The long-fruited melons are used much like cucumbers, fresh, pickled, or cooked. The round, familiar and much appreciated sweet melons are a later development, their origin having been traced to Central Asia of the ninth century (Paris et al. 2012b).

There are three species of pumpkins that today grown over much of the world (Nee 1990), all of which have 20 pairs of chromosomes ($2n = 2x = 40$). *C. pepo* L. is native to North America and has been cultivated there for 10,000 years (Smith 1995). *C. maxima* Duchesne is native to South America and, like *C. pepo*, favors temperate and sub-tropical climates. *C. maxima* has the largest fruits known, some having exceeded 1000 kg. *C. moschata* Duchesne, the tropical pumpkin, is probably native to northern South America, where it has been cultivated for 10,000 years (Piperno and Stothert 2003; Dillehay et al. 2007). *C. moschata* does well in the tropics, although some of its cultivars are adapted to temperate zones and others to semi-arid areas.

Squash are like pumpkins, belonging to the same three species, but instead of being round or nearly so, they have other shapes, usually elongated. Mature fruits are called winter squash and young fruits are called summer squash. The marked deviation of squash from the ancestral roundness of their wild gourd ancestors (Nee 1990) results in greater proportions of exocarp and mesocarp, and less endocarp, thus firmer, more pleasingly palatable young (summer squash) fruits (Sinnott and Durham 1929). *Cucurbita pepo* has eight fruit-shape morphotypes, of which the six deviating from roundness provide most of the summer squash (Paris 1986). The most popular morphotype has uniformly cylindrically shaped fruits and is called zucchini, also known as courgette (Paris 2000).

2. Reduction in yield and quality due to abiotic stress

Stress of plants or crops can be defined as the harmful impact of environmental conditions. Plants are dependent on their environment for optimal growth, and that environment may contain stresses, biotic and abiotic, that are detrimental to plant growth. Biotic stressors encompass pathogens and pests, and most of these are specific to particular crops or crop families. Abiotic stresses or unfavorable physicochemical environments, such as extremes in water availability and temperature, are considered to have a far greater adverse impact on crop production (Boyer 1982).

Abiotic stresses have been estimated to be responsible for 69% of yield loss in agronomic crops (Boyer 1982). Losses due to abiotic stress in horticultural crops, such as cucurbits, may be no less acute. Abiotic stressors are manifold and can affect the above-ground and the below-ground parts of the plant. Drought, salinity, extreme temperatures, lack or excess of minerals, herbicide residues, pollution, contamination, extreme pH, acid rain, flooding, and compaction can adversely affect the roots and their function. Extreme temperatures, high winds, air pollution, and natural disasters can directly affect the foliage, flowers, and fruits.

Of the various abiotic stresses, drought stress (lack of water) is the foremost (Boyer 1982; McCue and Hanson 1990; Cattivelli et al. 2008). In dry fields, irrigation is employed to prevent or relieve drought stress but irrigation often results, over time, in accumulation of salt in the soil, eventually resulting in saline stress (Cabot et al. 2014). Salinity is perhaps the second most important abiotic stress in crop production. Drought and salinity are insidious, disrupting osmotic and ionic equilibrium of plant cells (Mahajan and Tuteja 2005). Some stresses, including drought, salinity, heat, cold, and chemical pollution, tend to be interconnected and result in damage to cells by oxidation and osmotic imbalance (Wang et al. 2003). Plants that are salt tolerant are often drought tolerant and, interestingly, tend to tolerate other stresses, including excessively high and low temperatures (Zhu 2001).

Some primary causes of abiotic stress are easily identified in crop plants whilst others are less easily recognizable. The most obvious results of abiotic plant stress occur in large-scale farming, with crop plants. Severity of abiotic stress is a function of all of its possible causes and their interaction. Another viewpoint is from the response of the plant to various abiotic stresses. A plant's first line of defense against abiotic stress is its roots. If the soil holding the plant is healthy and biologically diverse, the plant will develop a larger and more efficient root system, thereby having a higher chance of thriving in spite of stressful conditions. Some mycorrhizae are known to benefit plants subjected to drought stress (Mo et al. 2016), including pumpkins (Harris-Valle et al. 2018).

Stress can be considered on an individual plant basis or on a crop basis. The same stresses affect individual plants as well as the crop as a whole. The exception is that individual plants are stressed by density, their number per unit area, meaning interplant competition for light, moisture, and nutrients. Wide spacing minimizes stress from this competition, allowing individual plants to develop optimally and produce maximum yield. However, maximum yield per individual plant almost always does not translate into maximum yield per unit area. The farmer wants maximum yield in his production area and chooses to sow or transplant at the optimal density of plants in his field or greenhouse accordingly. The optimal density for yield, and quality of yield, is a function of plant size and architecture (Donald 1963; Bleasdale 1966; Thompson 1966).

Abiotic stress factors can adversely affect both the quantity and the quality of yield. There are situations, though, in which relief of a stress factor on yield results in an adverse impact on quality, and vice versa. Horticultural crops, including fruit vegetables such as cucurbits, can react in various ways to abiotic stresses. Often, controlled abiotic stresses can be used to increase sugar content or improve nutraceutical value, but the timing of stress implementation is critical (Toscano et al. 2019).

Various abiotic stresses can adversely impact cucurbit crops around the world. Drought, salinity, cold and heat can be managed, to some degree, but at an expense to the grower and, in many areas, this expense is too great. Cucurbits have to compete for potentially highly favorable crop land with other potentially more lucrative crops as well as use by people for commercial development or other purposes (Wehner et al. 2020). Hence, cucurbits are often produced in less-than-optimal situations and will likely encounter stress over the course of the growing season (Villada 2015).

As with all crops, yield quantity and quality of cucurbits is determined by genetics, the environment, and management, and interactions among these three variables (Wehner et al. 2020). On the genetics side, some cultivars have a greater yield potential than others. Environmental components, mainly temperature and light intensity, restrict the particular crops to various regions of the world or reduce yield quantity and quality in those regions which have sub-optimal climactic conditions for the particular crop. Management includes the gamut from site selection and preparation, application of organic matter and fertilizer, irrigation, and especially the timing of their applications over the course of the growing season, to postharvest handling. Management practices adapted to particular regions and special crops can broaden the range of production of these crops. Special cultural techniques for particular cucurbits in different environments are almost as old as the crops themselves.

Cucurbits flourish in sunny locations with at least six hours of direct sunlight, rich, well-drained soil high in organic matter, and ambient air temperatures between 20 and 35 °C, the optimal around 25–32 °C (Wien et al. 2002; Wehner et al. 2020). Deviations from these ideals can stress cucurbit plants. The plants become spindly with inadequate lighting, weed competition, or if spaced too closely. They grow slowly with inadequate or excessive soil moisture, inadequate soil fertility, mineral concentrations, and organic matter, become chlorotic from excessive soil salinity or excessive soil mineral concentrations, seeds will not germinate and growth and fruit set is impaired by temperatures below 16 °C and above 40 °C, and the plants are killed by frost. Any of these less-than-optimal conditions can stress the plants and adversely affect the quantity and quality of cucurbit crop production.

Quantity of yield of cucurbits is a product of genetics, environment, and management and, especially, the interactions among them (Kyriacou et al. 2018). Some cucurbit cultivars have markedly higher yield potential than others, depending on region and season (Kumar and Wehner 2010–2011). However, in areas with short growing seasons, cultigens that bear earlier would have an advantage over those that could produce more in longer growing seasons (Loy 2004). Another example would be that many cultivars are well-adapted for production in the open field but less fit for production under protected conditions.

Cucurbit yields are a function of a multitude of other genetic, environmental, and management factors (Wehner et al. 2020). One management input, irrigation, is often necessary to achieve maximum crop yields, but can adversely affect the quality of mature cucurbit fruits; the timing of irrigation can be crucial to obtaining near-maximum yield without noticeable reduction in fruit quality. In a sense, irrigation is a management factor that can be necessary for optimal crop growth and production of mature cucurbit fruits but can also be considered a stress factor, if ill-timed, by resulting in decreased fruit quantity or quality. On the other hand, for production of immature cucurbit fruits, continual irrigation over the course of the growing season is a must. Cucumber and summer squash fruits are harvested several days after anthesis and have over 90% water content. After the first fruits are picked, cucumbers and summer squash plants can set more fruits. Sequential newly produced fruits are harvested every 1–3 days. For continued fruit set and production, the water lost to fruit growth at harvest has to be restored to the plants by repeated irrigation or rainfall. For continued growth, flower production, and set of quality fruits, the plants have to be harvested and irrigated frequently. Lack of water results in decreased production or its cessation.

Appropriate fertilization of cucurbit crops is a mixture of science, experience and art (Nerson 2008). Fertilization is a cultural practice that developed with farming. Various genetic, management and environmental factors complicate making the best decisions in the rates and timing of fertilizer and mineral application to cucurbit crops, including temperature and insolation. Moreover, optimal application regimes differ between field and protected conditions, with a basic application at planting and supplements during the growing season in the field contrasting with repeated, continuing applications through the irrigation system in protected conditions. With recent trends for development of environmentally sustainable agricultural systems, new research for optimal cucurbit fertilization and prevention of nutrient stress for these systems is much needed.

Quality of cucurbit crop yield is multifaceted. For mature cucurbit fruits such as watermelon, melon, pumpkin, and winter squash, the most outstanding of these traits is sweetness, often measured as soluble solids content, which is a close reflection of total sugar content (Tucker 1934; Thompson and Kelly 1957; Wehner et al. 2001; Magwaza and Opara 2015). Sugar content, though conditioned by genetic factors, is also influenced by environment and by field management. Irrigation too closely timed to harvest of mature cucurbit fruits can reduce soluble solids content (Wells and Nugent 1980; Kroen et al. 1991; Karchi 2000). Inadequate monitoring of fertilizer availability in the soil and inappropriate or poorly timed application of fertilizer can stress the plants and reduce fruit quality, and excessive weed growth results in strong competition for light, moisture, and nutrients (Hochmuth et al. 2001). For immature cucurbit fruits such as cucumber and summer squash, firmness and pleasant flavor without any bitterness are the most important aspects of fruit quality. For highest quality, the plants need to be irrigated repeatedly, as lack of water results in slower fruit growth and consequent reduction of fruit quality. Likewise, the fruits have to be harvested in a timely fashion, before they get too large and begin to lose their pleasant firmness and texture.

For all vegetable and fruit crops, consumer choice and purchasing are based mainly on appearance and quality. Ultimately, per capita consumption is based on consumer expectations from appearance and satisfaction from eating the product (Kader 2008). Appearance and eating quality are the basis of purchasing recurrence and loyalty, for a reasonable price (Kyriacou et al. 2018).

Quality in watermelon is first associated with appearance. Broad, dark stripes alternating with narrow light stripes, as exhibited by the diploid ‘Crimson Sweet’ and ‘Allsweet’, and by the many seedless triploid cultivars based on these two, are considered to be highly attractive in most markets. Further favorable visual impact is gained from intense color of the fruit flesh, especially red but also yellow. The attractive appearance must be accompanied by the physical texture of the flesh that imparts crispness and an absence of fibers, refreshing juiciness, and flavor largely based on sweetness (Maynard 2001). Ultimate eating quality anchored by a distinct, very attractive appearance is in itself a promotion that serves to enhance sales. A prime example and success of this approach is the diploid hybrid ‘Sangria’, which resembles ‘Allsweet’. ‘Sangria’ set a new consumer perspective of watermelon appearance and eating quality, its internal quality was said to have “a unique balance of flavor and texture along with high sugars and a deep red flesh” (Zanoni 2001). The red color of the flesh derives from the carotenoid pigment lycopene (Tadmor et al. 2005).

The most critical quality of watermelon is, of course, perceived sweetness (Kyriacou et al. 2018). Immature watermelon fruit-flesh is hard, white, and insipid. As the fruits develop and ripen, the fruit flesh becomes tender, and acquires color and sweetness, reaching a maximum at ripeness, which is usually between 28 and 40 days past anthesis (Wehner et al. 2001; Perkins-Veazie et al. 2012). Prior to ripening, the main sugars are the monosaccharides glucose and fructose. These are converted to sucrose as the fruit ripens. A minimum of 8% soluble solids is acceptable, though really good tasting watermelons have at least 10%, and excellence begins at 11%. For some of the best cultivars, such as ‘Crimson Sweet’, fruits having 13 and 14% soluble solids are not unusual.

Moderate drought stress induced by deficit irrigation, especially during the week or two prior to harvest, tends to increase watermelon fruit quality, though there are some disparate results that perhaps are due to varying cultural practices and climactic conditions, and perhaps particular cultivars (Kyriacou et al. 2018). Deficient irrigation of watermelons can increase vitamin C content of the flesh as compared with full irrigation (Kuşçu et al. 2015). Drought stress has also been implicated in increased content of citrulline (Davis et al. 2011), a non-proteinaceous amino acid that under drought increases tolerance to oxidative stress as a hydroxyl radical scavenger (Akashi et al. 2001). Citrulline, which also has several human health benefits, is especially abundant in watermelons (Hartman et al. 2019). Deficit irrigation also increases water use efficiency of the watermelon crop (Qin and Leskovar 2020).

Quality in melon is largely measured by soluble solids (sugar) content of the fruit flesh. Sugar content in mature melon fruits is mostly sucrose (Burger et al. 2000). The amount of sucrose accumulated in the fruit flesh is a function of the rate of sucrose accumulation and the length of the sucrose accumulation period (Schaffer et al. 2000) and, as such, is highly subject to genetic and environmental factors. Appearance and texture is another aspect of mature melon fruit quality. Texture components include firmness, “melt-in-the-mouth”, density, juiciness, and stringiness (Guérineau et al. 2000). Other aspects include appearance, density, and aroma. Harvest date can be critical to obtaining optimal quality of the product, and can differ widely among various cultivars and growing areas (Guérineau et al. 2000). Also, like other vegetables and fruits, as the time between melon harvest and eating increases, the quality of the product decreases (Kader 2008).

Melons, like watermelons, do not accumulate sugars after harvest. Melons show a wide range of tolerance to drought stress (Karipcin et al. 2016). Moderate drought stress of melons induced by deficit irrigation tends to decrease yield but can improve the quality, the amount and timing of irrigation being critical for attaining the optimal balance between them. Yields are often reduced by over 20%, but soluble solids content of the fruit flesh can be increased by over 20% and of beta-carotene content by as much as 25% (Sharma et al. 2014). Among vegetable crops, melons are considered to be moderately tolerant to salt stress, and there is a rather wide range of tolerance to soil salinity in melon germplasm. Tolerance to salinity can be measured as a percentage of the values obtained at the control (considered non-saline) level. Melon plants can be stressed when salinity exceeds threshold levels which, in turn, are dependent on stage of plant development. Nonetheless, reduction in leaf area of seedlings due to salinity was observed to be a good predictor of reduction in yield (Nerson and Paris 1984; Franco et al. 1997). Salinity typically reduces yield in melon but, at moderate levels, can improve quality by increasing soluble solids content of the fruit flesh (Shannon and François 1978; Mendlinger 1994). Melons are more sensitive to cool temperatures than cucumbers. Nonetheless, there is germplasm that is capable of germinating well at below what is considered the threshold temperature for germination, 16 °C (Nerson et al. 1982).

Quality of pumpkin and winter squash is largely determined by the amounts of starch and sugar, and balance between them, in the fruit flesh (Loy 2004). Appearance, consistency (firmness, texture components), density, flavor, and aroma also play major roles (Culpepper and Moon 1945). Unlike watermelons and melons, pumpkins and squash accumulate starch in the fruit mesocarp, which is gradually converted to sugars both pre- and postharvest.

Pumpkin and winter squash fruit can be considered to have three overlapping developmental phases, expansion, dry matter accumulation in the mesocarp, and seed maturation (Loy 2004). Maximum fruit size is achieved within three weeks after anthesis in small-fruited cultivars, but after four weeks or more in large-fruited cultivars. The dry matter accumulated in the mesocarp is mostly starch, and the mature seeds have an oil content of 40% or more. Dry matter accumulates linearly from 10 to 30 or even 40 days after anthesis, then gradually drops off, with starch being converted to sugars. Fruits need to have at least 15% dry mass and 10% soluble solids to have generally acceptable quality. Fruits harvested at 30 days past anthesis or less have not yet developed near-maximum sugar content or the consistency to be generally liked by consumers. At ripening and in storage, increases in amount of sugars occur at the expense of fresh weight. Thus, attempts at breeding for increased yield as measured by fresh weight may result in reduction of fruit quality.

Quality of summer squash is largely influenced by the timing of harvest, which should be no more than five days past anthesis (Loy 2004). Older fruits are less succulent, losing their moist, firm, smooth “melt-in-the-mouth” appeal and are a drain on plant resources. Larger fruits also have less proportion of attractive exocarp and firm mesocarp, and greater volume of the placenta and seeds, thereby rendering them unacceptable. Summer squash are approximately 6% dry weight (Lorenz 1949; Gebhardt and Thomas 2002) and fruit number is a better indicator of yield than fresh weight because younger, smaller fruits tend to fetch higher prices at markets than larger ones.

Summer squash plants are indeterminate and will exhibit continuous growth and flowering (El-Keblawy and Lovett-Doust 1996), provided that they are harvested at least three times weekly to ensure, as much as possible, that all the fruits they produce are harvested on time. Fruits that are ready to harvest but missed because of the dense foliage become too large and old, sapping strength from the plants; these fruits have to be picked from the plants and discarded to allow future regrowth. Summer squash crops are usually harvested for 3–6 weeks in the field or several months in the greenhouse. Throughout the harvest period, quality of the fruits can be adversely affected by abiotic factors that result in their slower growth, including drought stress, extreme temperatures, and inadequate or excessive amounts of elements in the soil or medium. Postharvest, the fruits are subject to desiccation and chilling injury (Hruschka 1977; Sherman et al. 1987; Rodov et al. 2020).

Quality of cucumber, like summer squash, is also largely influenced by the timing of harvest. Larger fruits have less proportion of attractive exocarp and firm mesocarp, and greater volume of the placenta and seeds. Fruits that are too old are softer and less crisp, and the seeds within are larger and harder, which is objectionable to the consumer.

Cucumbers have 4% dry weight and are less nutritious than summer squash (Gebhardt and Thomas 2002). However, like summer squash, cucumber plants are indeterminate and will exhibit continuous growth and flowering, provided they are harvested frequently enough to prevent development of older, larger fruits. Field-grown plants can be harvested for weeks and greenhouse-grown plants for months. Throughout harvest, quality of fruits can be adversely affected by abiotic factors that slow growth, such as drought, temperature extremes, and inadequate or excessive fertilizer or contamination of the soil or medium. Postharvest, the fruits are subject to desiccation and chilling injury. Requirements for fruit quality of cucumbers for processing can be stringent (Staub et al. 2008). Mesocarp and seed cavity discoloration, hollow placenta and carpel separation are particularly undesirable and can be dramatically affected by growing environment. Overall, there is much genetic variation among cucumber accessions for tolerance to abiotic stresses but complex environmental interactions have made breeding for improved stress resistance in cucumber expensive and challenging.

3. Growing importance in the face of climate change and increasing population

Average global temperatures have been increasing steadily since the middle of the twentieth century and are expected to continue to increase over the course of the twenty-first century. This change in climate can pose a serious threat to human health (Anderson and Bell 2009; Shi et al. 2016). Increasing temperatures are also a major threat to maintaining crop yield and quality.

The largest adverse impacts of global warming on human populations are expected to be increased mortality due to heat stress, increased mortality and sickness from vector-borne (such as malaria) and water-borne (such as cholera) diseases, decreased drinking water availability in semi-arid areas, greater risk of flooding in low-lying and coastal areas, and reduced crop yields in tropical and sub-tropical regions due to excessively high temperatures (Population Reference Bureau 2001). On the other hand, global warming can have good impacts by reducing winter mortality in mid- and high-latitude regions and allowing expansion of agricultural production to these regions.

The largest threats to crop production from global warming relate to water availability and temperature (Ferguson 2019). Climate change is increasing the impact of drought stress and heat stress. A large proportion of world agriculture is conducted in seasonally arid regions, which tend to be subject to volatile environmental and climactic conditions. Global warming can be expected to strongly impact these regions and further destabilize agricultural production. Together with the increasing world human population, the continuing ability to produce sufficient food and sustenance will be a challenging endeavor.

Crop breeding has been called upon to address this challenge. One approach will be the need to tap wild and primitive relatives of crops for genes that confer resistance to stress, and a plan for the identification of missing germplasm, collection and evaluation of novel germplasm, and information distribution has been proposed (Dempewolf et al. 2014, 2017). Domestication has over the millennia narrowed the genetic base of our crop plants by adapting them for optimal production and quality under cultivation. This long process has reduced allelic diversity and resulted in loss of potentially valuable variants for adaptation to stressed environments. These valuable genes may still be found in the gene pool of primitive or wild relatives of crop plants. Another approach will be the need to better understand the physiology and genomics of abiotic stress resistance, especially drought stress.

There is a fairly comprehensive understanding of the physiological mechanisms that facilitate the maintenance of productivity in response to drought, flooding, and heat stress. QTLs (quantitative trait loci) have been identified for tolerance. Consequently, foundations have been laid to begin more efficient development of crop varieties primed for climate change (Tuberosa and Salvi 2006; Ferguson 2019). These two approaches need to be applied together to more efficiently breed and deploy stress-tolerant elite crop varieties.

Generally, cucurbits are best adapted for production in warm regions. The wild ancestors of the five major cucurbit crops hail mostly from seasonally arid parts of the Americas (pumpkins and squash), Africa (watermelons) and Asia (melons and cucumbers) (Paris 2016a, b). Given their ancestry, it is not surprising that some cucurbit crops are considered to be moderately tolerant to drought stress and salinity (Karipcin et al. 2016) and are even cultivated under “dryland” conditions in semi-arid areas (Karchi 2000). Nonetheless, climate change and global warming can be expected to result in extended periods of excessive heat in traditional cucurbit-growing areas, subjecting cucurbit crops to stress which will impact as a marked reduction in their production. Even today, for example, excessively high temperatures inhibit female flower differentiation, development, and fruit set of cucurbits (Wien et al. 2002). Higher temperatures will also increase crop requirements for irrigation to maintain growth and production.

4. Limitations of traditional breeding and rationale of genomic designing

Improvements in the adaptation of plants to abiotic stress can make major contributions to agricultural production but faster progress would occur if the fundamental mechanisms of adaptation to abiotic stress are better understood (Boyer 1982). Over the centuries, the practice has been to improve crop productivity in conducive environments and to breed for greater and higher quality productivity in these environments. This approach has been highly successful. Often, though, environmental factors are adverse to optimal yield and quality.

Drought is considered to be by far the most significant environmental stress in agriculture worldwide (Cattivelli et al. 2008). Soil salinity is also significant in many areas, especially in irrigated agriculture (McCue and Hanson 1990). Tending to be concomitant with these stresses is high-temperature stress (Wang et al. 2003). Tolerance to all three of these stresses are major objectives in some plant breeding programs. Breeding for increased yields in optimal environments has increased yields in stress conditions, to some extent. Further progress would require reduction of the gap between yields in optimal environments and yields in water-limited environments (Cattivelli et al. 2008).

Crop evolution under domestication has been focused on increasing yield and quality. In the wild crop ancestors, natural selection favored adaptation, reproduction, and survival. Crop wild relatives, therefore, may harbor genes for adaptation to diverse, stressed environments. Crop relatives can potentially improve adaptive capacity of crops in various climates and agricultural management systems (Dempewolf et al. 2017). They can contain a pool of genetic diversity from which to draw new allelic variation in the crop plant taxon, including tolerance to abiotic stresses.

For further progress in breeding for abiotic stress tolerance, sources of recently acquired knowledge are expected to be useful (Cattivelli et al. 2008), mainly new insights from plant physiology on the network of drought-related traits and quantitative trait loci (QTLs) that affect expression of drought-related traits (Tuberosa and Salvi 2006). Breeders would need to lead the way to apply this knowledge. However, integrating and developing an effective breeding program, the ultimate goal of which would be to develop cultivars with both, high yield potential and high yield stability for dry or saline conditions, would not be an easy task. This is because assessment of drought tolerance among various accessions needs to be based on physiological parameters related to drought stress and identifying parameters best suited for such an assessment. In the end, yield performance would be the bottom line, requiring testing across a range of soils and environments. Ideally, a drought-tolerant cultivar needs to possess a strong stress-response capability that does not impinge on crop performance in more optimal environments (Wang et al. 2003). Conventional breeding and selection techniques will continue to make an essential contribution to these efforts.

Breeding efforts to improve plant tolerance to drought, high salinity and extreme temperatures are hampered by the genetic complexity of stress responses (Cushman and Bohnert 2000) as well as low heritability, complex genetic control, and genotype-by-environment interactions. Comparative genomics of diverse plant taxa, using the newest technologies, could the result in the discovery of novel genes controlling adaptation to stress and their mechanism of action, and even indicate possible methodologies for their deployment in horticulturally advanced germplasm.

The five cosmopolitan cucurbit crops are annuals, with a life cycle of 3–4 months, and are derived from wild ancestors growing in warm, seasonally arid regions (Paris 2016a, b). Given their ancestry, they would be expected to have greater tolerance than other vegetables to drought, salinity, and high temperatures, which is indeed the case. Cucurbit plants, though, are herbaceous and large, requiring uptake of sufficient amounts of water to maintain themselves and grow and produce flowers and fruits (Wien 2002; Wehner et al. 2020). The large size of cucurbit plants limits the amount of plants that can be grown in a given amount of land area. Thus, much land area is required to grow segregating populations intended for selection of any desired trait, such as abiotic stress tolerance. Cucurbit plants are self-compatible and, as the flowers are relatively large, they are quite easily handled for controlled pollination. The flowers open in the early morning and are functional for only several hours after opening, withering thereafter. The outside of the corolla yellows on the day before anthesis. Controlled pollinations can be conducted by clamping shut these flowers at that time, and making the controlled pollination on the day of anthesis by plucking off the male flower and bringing it to the female flower, and making contact between the anthers and the stigma. The female flower is then immediately clamped shut again, and a tag tied to its stem indicating the pollinator and date of pollination. If the flowers are hermaphroditic rather than female, their anthers have to be removed on the day before anthesis. A single fruit can produce several hundred seeds. Typical breeding strategies include backcrossing, selfing, and cycles of backcrossing and selfing. Backcrossing is employed to introduce a simply inherited trait, such as resistance to a particular disease. Pedigree breeding is employed for traits of complex inheritance, such as yield and quality, and backcross-pedigree breeding for complex or recessive traits (Allard 1960). The environment, macro- and micro-, can play havoc in trying to select plants that are genetically stress tolerant. Success of breeding for complex traits can only be determined by testing and comparing progenies, requiring much in the way of resources including land, agricultural inputs, and manpower.

Cucurbits, like other crops, can be stressed by biotic and abiotic factors. *Cucurbit Diseases*, a compendium complete with descriptions and quality photographs, presents the results of disease infections and abiotic stresses on cucurbit plants and fruits, the abiotic stresses including air pollution, nutrient deficiencies, temperature and moisture extremes, pesticide injury, and salinity (Petoseed 1988). For all of the abiotic stresses, horticulturally acceptable resistant or tolerant cultivars were stated to be unavailable. Even to the present, cucurbits lag behind other important crops in the discovery of genes conferring abiotic stress tolerance, and little effort has been focused on this subject (Wehner et al. 2020).

Of the 62 morphological and phenotypic genes in the 2012 Gene List for watermelons (Wehner 2012), only one gene for abiotic stress tolerance is listed. *Ctr* is a dominant gene for *Cool temperature resistance*. Watermelon accessions differ greatly among one another in tolerance to drought stress (Wehner 2008; Zhang et al. 2011).

Melon accessions vary for abiotic stress tolerances. Of the 159 morphological/phenotype genes described in the latest Gene List for melons (Dogimont 2010–2011), there is not even a single one listed for any kind of abiotic stress tolerance. Nonetheless, three or more recessive genes and a cytoplasmic factor have been reported to confer low-temperature germination in melon (Hutton and Loy 1992). Melon accessions differ in tolerance to drought stress (Karipcin et al. 2016), salinity (Nerson and Paris 1984; Franco et al. 1997), and cadmium stress (Zhang et al. 2016). There is also another report that melon accessions differ in their ability to germinate at sub-optimal temperatures (Nerson et al. 1982).

For pumpkins and squash, substantial ecogeographical variation was observed both within species and among species, to drought stress and low temperatures (Khoury et al. 2020). The genus *Cucurbita* contains several xerophytic species as well as the cool-tolerant *C. ficifolia* Bouché. However, in the Gene List for *Cucurbita* species (Paris and Kabelka 2009), only one gene is listed that is connected with a disorder that is exacerbated by drought stress (Paris et al. 1993). This is the recessive gene *sl*, for silverleaf resistance (Gonzalez-Roman and Wessel-Beaver 2002; Young and Kabelka 2009).

Of the 199 genes listed and described in the latest Gene List for cucumber (Weng and Wehner 2016–2017), there are only four for abiotic stress resistance. One of them is a dominant gene for salt tolerance, *Sa*, identified in USDA PI 177361. Another, tolerance to the air pollutant sulfur dioxide, is the dominant gene *Sd* from 'National Pickling'. Seedling chilling resistance is conferred by the dominant gene, *Ch*, from breeding line NC-76, which was derived from USDA PI 246930. Waterlogging tolerance is conferred by the QTL designated qARN6.1. There is much reported variability in cucumber germplasm for tolerance to abiotic stresses (Staub et al. 2008). Tolerance to low-temperature stress at germination varies among accessions but has low to moderate heritability, requiring selection based on families rather than single-plant selection (Nienhuis and Lower 1981; Wehner 1982). Likewise, cucumbers exhibit genetic variation for low-temperature stress of seedlings, but tolerance to this stress has low heritability and requires selection from replicated progenies rather than single plants (Wehner and Kozik 2007). Cucumber accessions differ among themselves in germinability and seedling development under drought stress, too (Klosinska et al. 2016). Abiotic stresses of herbicide damage, drought stress, cold germination, heat damage, chilling damage, and saline stress were listed in a survey of public-sector cucumber research (Weng 2008–2009). The mode of inheritance of tolerance to most abiotic stresses is unknown, likely complex and influenced by various other factors pre- and postharvest, and thus traditional breeding for stress tolerance has been time-consuming and expensive. Traditional breeding for stress tolerance, though, has had some success. A prime example is furnished by "pillowy" fruit mesocarp, which is caused by drought stress and associated with low calcium levels, can be exacerbated by temperature and humidity. Drought stress can also result in cessation of plant growth and, as cucumbers are picked continually over the course of the growing season, result in complete cessation of fruit production.

Interspecific crossing of cucurbit crop taxa has long appealed to cucurbit breeders, by offering possible options for assimilating desirable characteristics into horticulturally advanced cucurbit germplasm, including tolerance to abiotic stress. Although many such attempts have been made with *Cucumis melo*, melon, no such crossing has as yet been reported to result in introgression of a character of horticultural interest (Pitrat 2008, 2017). *C. sativus*, cucumber, is quite isolated from other *Cucumis*, the nearest relative being *C. hystrix* Chakr. (Staub 2008). Some interspecific crosses have been successful among the various dozen species of *Cucurbita*, which have allowed introgression of disease resistance from one species into another (Ferriol and Pico 2008). Difficulties have been encountered in almost all cases, ranging from "quasi-linkage" or "genetic drag" in backcross progenies to sterility of interspecific F_1 plants. Crosses between the three cosmopolitan *Cucurbita* species with the xerophytic species, which would be excellent sources of resistance to drought, have been a complete failure. Barriers to interspecific crossing among the species of *Citrullus*, specifically with *C. lanatus*, the dessert watermelon, are much weaker than those encountered in *Cucumis* and *Cucurbita* (Levi et al. 2017). The citron watermelon, *Citrullus amarus* Schrad., and the colocynth, *Citrullus colocynthis* (L.) Schrad., provide sources of resistance to biotic and abiotic stresses for dessert watermelon and, even though difficulties involving quasi-linkage and sterility are encountered in interspecific crosses, these have been largely surmountable (Levi et al. 2006a, b; Levi et al. 2011). Some citron watermelon accessions produce much larger root systems than dessert watermelons (Katuuramu et al. 2020), thus offering a infrageneric source of tolerance to drought stress. The closest relative of the dessert watermelon, *Citrullus mucosospermus* (Fursa) Fursa, is an underexploited possible additional source of stress tolerance (Chomicki and Renner 2015; Levi et al. 2017).

Genomic designing of crop plants is a planned manipulation of the genome for the purpose of improving plant output or performance. Plant response to abiotic stress, such as drought, is complex and multigenic, and quantitative genetics together with genomics technologies may pave the way for identifying individual genes and regulatory loci and their network of interactions at all levels of function (Habash et al. 2009). The complete genome sequences of numerous organisms has been revealed by next-generation DNA sequencing technologies (Haimovich et al. 2015). Designed engineering of the genome aims to introduce targeted genomic changes and its expansion is expected to enable targeted modifications, some of which may elucidate the causal links between genotype and phenotype, and enable redesign of organisms. Genome editing tools are available and provide opportunities for introducing targeted genome modifications efficiently (Jain 2015). Nonetheless, in the field, plants are often subjected to multiple stresses which can vary in duration and intensity, and therefore the performance of genetically modified genotypes with purported stress tolerance must be evaluated under realistic agricultural conditions (Cominelli et al. 2013).

Over the past 20 years, there has been great progress in genomic technologies and the genomes of cucurbits have been scrutinized with these new technologies (Grumet et al. 2017). Cucurbit crops tend to have small genome sizes, between 350 and 450 Mbp, and draft genome sequences have been assembled for cucumber, melon, watermelon, squash, and pumpkin. These assemblies have made possible determining syntenic relationships among cucurbit genomes (Nimmakayala et al. 2017) as well as the identification, characterization, and location of genes in the genome (Grumet et al. 2017). Some efforts have been focused especially on genes conferring resistance to diseases and insects. However, there does not seem to have yet been a sustained genomic effort in the cucurbits to identify, characterize, and localize genes that confer abiotic stress tolerance. If it is possible to localize regions in the genome that are responsible for increased tolerance, genomic selection may well prove to be a methodology for quicker and more efficient selection of tolerant genotypes.

The genomes of the five major cucurbit crops have been sequenced and re-sequenced (Casacuberta et al. 2017; Montero-Pau et al. 2017; Weng 2017; Guo et al. 2019; Wu et al. 2019; Xanthopoulou et al. 2019; Zhao et al. 2019; Cucurbit Genomics Database 2020). So the stage appears to be set for attempting to genetically modify cucurbits for tolerance to abiotic stress. The chapters following will describe aspects of various abiotic stresses, offer glimpses on classical genetics and traditional breeding of cucurbits, briefly touch on diversity analysis, as well as describe association mapping, marker-assisted breeding for stress tolerance, genomics-aided breeding for stress tolerance, recently developed concepts and strategies, and briefly touch on genetic modification for stress tolerance as well as making an accounting on the possible use of bioinformatics, capped by a look to the future of expanding cucurbit crop production.

6.2 Glimpses on Classical Genetics and Traditional Breeding

1. Classical mapping efforts (morphology and isoenzyme)

The five cosmopolitan cucurbit crops show broad phenotypic diversity. Nonetheless, investigations of the mode of inheritance of these traits has lagged behind that of other horticultural crops (Wehner et al. 2020).

Watermelon, *Citrullus* Schrad., has 11 pairs of chromosomes ($2n = 2x = 22$). There are seven species in the genus, the most familiar being the dessert watermelon, *C. lanatus* (Thunb.) Matsum. & Nakai. Others, less commonly cultivated, are *C. amarus* Schrad. (citron watermelon), *C. colocynthis* (L.) Schrad. (colocynth), and *C. mucosospermus* (Fursa) Fursa (egusi watermelon), all of which can be intercrossed, with some impairment, to the dessert watermelon. Near the end of the twentieth century, 103 of the 153 listed gene loci for watermelon were variant isozyme alleles (Rhodes and Dane 1999). Most of the reported linkages in watermelon were for isozyme loci, which were distributed into seven linkage groups (Navot and Zamir 1986; Navot et al. 1990). Significantly, two genes conferring important phenotypic traits, a dominant gene *Bi* for *Bitter fruit* (versus non-bitter) and a recessive gene *red* for *red fruit-flesh color* (versus white) color were found to be linked to two isozyme loci that are in the same linkage group (Navot et al. 1990). By 2003, the number of listed gene loci increased by only 10, to 163, with no new information concerning linkage (Guner and Wehner 2003) and by 2007 by only eight to 171, again with no further linkage information (Wehner 2007). Even though genes affecting such diverse traits as vine length, sex expression, fruit shape, rind toughness and color pattern, flesh color, seed size and color, resistance to insect pests, nematodes, fungal, bacterial, and viral diseases, and cold temperature tolerance had been identified (Gusmini and Wehner 2005–2006), no further cases of linkage were reported.

Melon, *Cucumis melo* L., has 12 pairs of chromosomes ($2n = 2x = 24$). Near the end of the twentieth century, only eight linkage groups had been proposed for the genes of melon. At that time, 120 loci were known for melon, including 16 isozyme loci (Pitrat 1998). Some loci affecting phenotypic traits were assigned to eight linkage groups, with Groups 1, 5, 7, and 8 having two loci each, Groups 2 and 6 with three loci each, Group 3 with four loci, and Group 4 with five loci (Pitrat 1994). Six of the isozyme loci formed a linkage group and five others formed another linkage group (Staub et al. 1998). The 2006 Gene List for melon presents 170 loci, of which 28 were for isozyme variants (Pitrat 2005–2006). However, allelism tests have been lacking, possibly inflating the number of gene loci. No further linkages were reported.

Cucumber, *Cucumis sativus* L., has only seven pairs of chromosomes ($2n = 2x = 14$). Near the end of the twentieth century, only six linkage groups had been proposed for the genes of cucumber, with recombination frequencies mostly lacking (Robinson and Decker-Walters, 1995). At that time, 146 loci were known for cucumber and, 20 years later, 167 loci were identified (Wehner et al. 2020). The 2005 Gene List for cucumber presents 167 loci, of which 21 were for isozyme variants (Wehner 2005–2006). Many of the genes were assigned to one of six linkage groups, with Linkage Groups A and D having 12 genes each, B having nine genes, C four genes, E three genes, and F two genes. Later, 10 pairs of genes, including three new ones, were found to be linked (Wehner et al. 2018).

Pumpkin and squash, *Cucurbita* L., have 20 pairs of chromosomes ($2n = 2x = 40$). There are approximately 12 species in the genus, the most widely distributed in cultivation being *C. pepo* L., followed by *C. maxima* Duchesne and *C. moschata* Duchesne. There are two other cultivated species, *C. argyrosperma* C. Huber and *C. ficifolia* Bouché, and approximately seven other species that grow wild in North America or South America but are not cultivated. Near the end of the twentieth century, 102 loci were listed for *Cucurbita*, 68 for phenotypic traits and 34 for isozyme variants (Hutton and Robinson 1992; Robinson and Hutton 1996; Robinson and Paris 2000). No information was provided concerning possible linkages. Much-expanded gene lists from the next decade present 78 loci for phenotypic traits and 48 isozyme variants (Paris and Brown 2004, 2005). Three cases of linkage are named, between genes *D* and *mo-2* and between genes *M* and *Wt* in *C. pepo* and between genes *Bi* and *Lo-2* in a cross between *C. ecuadorensis* Cutler & Whitaker and *C. maxima* (Paris and Brown 2004).

Clearly, gene mapping in cucurbits via classical breeding has not been highly efficient. Knowledge of placement of genes among the chromosomes, even for cucumber with its seven pairs of chromosomes, was extremely limited until the advent of molecular genetics and its application to mapping.

2. Limitations on classical endeavors and utility of molecular mapping

Cucurbit plants are herbaceous annuals with a life cycle of 3–4 months, sprawling over a distance of a meter or more in every direction (Wehner et al. 2020). A lot of space is required to raise a segregating population that is large enough to identify the mode of inheritance of most phenotypic traits. Even more space would be required to raise populations segregating for more than one gene in order to determine whether the genes are linked. Hence, classic approaches to gain knowledge of genetic linkage in cucurbits have not been very fruitful.

Growing cucurbits for breeding and genetics research, though requiring much space, is relatively straightforward. Controlled pollinations need to be conducted by hand because the plants are naturally both cross- and self-pollinated (Wehner et al. 2020). The plants are self-compatible and the flowers are relatively large and thus easily manipulated. Cucurbits usually do not suffer from inbreeding depression but hybrids between genetically distant parents show marked heterosis. Controlled pollinations, both selfing and crossing, are easy to conduct. Ordinarily, pumpkin, squash, and cucumber plants are monoecious, melons are usually andromonoecious, and watermelons may be monoecious or andromonoecious. Anthesis occurs just before dawn and the flowers are open and functional for several hours, until mid- to late morning, of a single day, withering under the heat of the day. As the outside of the apical part of the corolla yellows on the day before anthesis, flowers primed to open the next day can be recognized and prepared for controlled pollination. The five petals are fused at the base, separating from about halfway to the apex. Thus, the apical part of flowers intended for controlled pollinations are clamped, tied, or covered shut on the day prior to anthesis in order to prevent the entry of bees or other potential pollinating insects into the flower. The next morning, the pollination is accomplished by plucking off the male flower and bringing it to the female flower. The pollen is sticky and the pollination is accomplished by merely making contact between the anthers and the stigma. The female flower is then immediately re-secured, and a tag tied to its stem indicating the pollinator and date of pollination. However, if the pistillate flowers are hermaphroditic rather than female, their anthers have to be excised on the day before anthesis, the excision requiring whole or partial removal of the corolla. Most melons and some watermelons are andromonoecious, and thus controlled cross-pollinations with them are laborious. A single fruit can produce several hundred seeds. Typical breeding strategies include backcrossing, selfing, and cycles of backcrossing and selfing. Backcrossing is employed to introduce a simply inherited trait, such as resistance to a particular disease. Pedigree breeding is employed to traits of complex inheritance, such as yield and quality, and backcross-pedigree breeding for complex or recessive traits (Allard 1960). Success of breeding for complex traits can only be determined by testing and comparing progenies, requiring much in the way of resources including land, agricultural inputs, and manpower.

Fruit size and shape are under multigenic control. Wild relatives of cucurbit crops have small, round fruits and cultigens have larger fruits which may deviate noticeably from roundness. The action of each gene for increased size or deviant shape is thought to be geometrically rather than additively incremental (Sinnott 1937). Other horticultural traits of utmost importance, earliness, yield and fruit quality, have more than one component and are also under complex multigenic control. Nonetheless, some genes for earliness and productivity have been identified, such as the *Bu* (*Bush*) gene in pumpkin and squash (Ferriol and Pico 2008) and the *F* (*Female*) gene in cucumber (Staub et al. 2008), and for higher fruit-flesh quality, the *suc* (*sucrose*) gene in melon (Burger et al. 2002).

Traditional breeding approaches have efficiently isolated novel mutants and have produced novel genetic variants, through self-pollination, hybridization, pedigree selection, and backcross-pedigree methodologies (Allard 1960). Traditional breeding, though, is often time-consuming, dependent on generation time, and difficulties are encountered in attempting to cross distant relatives. Often, with conventional methodologies, pyramiding more than a few desirable traits into a single elite accession requires growing out more generations and/or larger populations in each generation, along with being able to clearly distinguish each desired trait in individual plants, or progeny test the individual plants to determine if any particular desired trait has high heritability. For this kind of plant-breeding situation, especially, molecular mapping can be quite useful.

Before the advent of molecular techniques, genetic maps were based solely on variant alleles producing a detectable phenotypic or morphological effect. Construction of genetic maps was an arduous task undertaken over a period of many years. A large number of genetic loci affecting phenotype were identified for some intensely studied organisms, including such crop plants as *Zea mays* L. (maize) and *Solanum lycopersicum* L. (tomato). However, even in these organisms there were long chromosome intervals between genes that had large amounts of DNA, that is, these maps were far from being saturated. Additional markers were needed to fill in the large gaps.

The first type of molecular marker to be used for mapping in cucurbits was isozymes (Wang et al. 2006). Isozymes are variants of the same enzyme having the same or similar functions. They are recognized by having differing electrophoretic mobility. Allelic variation at isozyme loci is called allozymic polymorphism. Allozymes make possible comparisons among individuals in a population and are inherited in Mendelian fashion with codominant expression. They were commonly used as markers to supplement genetic maps (Weeden and Wendel 1989). As noted above, most of the reported linkages in watermelon until 1990 involved isozyme loci, distributed into seven linkage groups and two of the isozyme loci were assigned to the same linkage group as two genes conferring important phenotypic traits, *Bi* for *Bitter fruit* (versus non-bitter) and *red* for *red fruit-flesh color* (versus white) (Navot and Zamir 1986; Navot et al. 1990). For melon, 11 isozyme loci were found to be linked into two groups but no linkages were reported between any of them and phenotypic markers (Staub et al. 1998). For cucumber, which has only seven pairs of chromosomes, 18 polymorphic isozyme loci were found to be in the same three linkage groups as 12 morphological loci (Meglic and Staub 1996). For pumpkin and squash, development of molecular mapping has lagged behind that of the other three cosmopolitan cucurbit crops (Brown 2001). One isozyme marker was linked to a gene conferring virus resistance (Weeden et al. 1984). Ten other isozyme loci were observed to be linked, distributed into five linkage groups (Weeden and Robinson 1986a, b).

A more efficient approach for constructing genetic maps was developed by making possible the detection of polymorphisms in length of homologous DNA fragments (Tanksley 1988). These molecular markers are defined as "sites of heterozygosity for some type of silent DNA variation not associated with any measurable phenotypic variation" (Griffiths et al. 2000). A DNA marker is a small, particular region in a sequence showing polymorphism by insertion, deletion, or substitution, among individuals (Jiang 2013a, b).

DNA markers are numerous in the genome and are easily detected (Griffiths et al. 2000). By linkage analysis, they can be mapped in relation to each other as well as to loci that affect the phenotype. These markers, though lacking or of unknown biological significance, act as convenient reference points for orientation in the genome. They first began to be used in cucurbits in the 1990s (Wang et al. 2006).

One of the first of the molecular techniques deployed in higher organisms was named restriction fragment length polymorphism (RFLP) analysis (Tanksley 1988). The segregation of markers obtained by using this technique can be monitored in generations obtained from controlled crossing and the markers assigned to linkage groups and mapped according to standard genetic analysis. RFLP analysis has been largely superseded by more recently developed techniques because it demands large amounts of DNA, the results obtained among different laboratories are difficult to reconcile, and the procedure is too laborious (Jiang 2013a, b).

Another early molecular technique was named random amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990). Using this relatively quick, simple technique, polymorphisms are observed as DNA segments which amplify from one parent but not the other. They, too, can be used to construct genetic maps. However, RAPD markers have low reproducibility and most are dominant, therefore incapable of detecting heterozygotes (Jiang 2013a, b).

More recently developed DNA-based markers have important advantages (Jiang 2013a, b). One of these is amplified fragment length polymorphism (AFLP) analysis. AFLPs are reliable, with stable responses to small variations in DNA amplification procedures, reproducible, and have high marker density. However, high-quality DNA is required and marker development is complicated. Another is simple sequence repeat polymorphism (SSR), also called microsatellite, analysis. These are random, tandem repeats of nucleotides, 2–6 base-pairs long, and are a source of polymorphism as the number of copies varies among individuals. They are highly polymorphic, reproducible, and are co-dominant. However, their development is labor intensive.

Over time, RFLPs, RAPDs, AFLPs, and SSRs have been used in cucurbits for mapping and assessment of genetic diversity. Others used in cucurbits include inter simple sequence repeats (ISSRs), sequence-related amplified polymorphisms

(SRAPs), cleaved amplified polymorphic sequences (CAPs), expressed sequence tags (ESTs), sequence characterized amplified regions (SCARs), high frequency oligonucleotides: targeting active genes (HGO-TAGs), and single nucleotide polymorphisms (SNPs). Overall, SSRs seem to have the greatest concentration of desirable features and are thus the marker of choice for deployment in marker-assisted selection in many crops (Jiang 2013a, b). Moreover, recently, whole-genome sequencing has provided sequence information, facilitating the development of SSR markers.

The utility of molecular markers in breeding programs is most obvious in particular situations (Jiang 2013a, b). The advantages of molecular breeding are greatest when phenotyping is difficult or expensive, target-trait heritability is low, expressivity and penetrance of the target trait is highly affected by the environment, the trait is expressed relatively late in the plant life cycle, and when pyramiding of genes for enhanced trait expression is required (Das et al. 2017).

Molecular mapping of quantitative trait loci (QTLs) is especially challenging (Jiang 2013a, b). Most of the important horticultural or agronomic traits, such as yield, are polygenic and controlled by multiple QTLs, with each gene having a small phenotypic effect. Phenotyping is difficult, as is identifying any phenotype-marker association. Progeny testing is required, preferably at multiple sites or in multiple years. Environmental and epistatic interactions further complicate obtaining a correct assessment of a phenotype-marker association.

Prerequisites, activities, procedures, and theoretical and practical considerations for utilization of molecular, that is, marker-assisted breeding, are described in detail by Jiang (2013a, b). Required of the molecular markers would be reliability, co-dominance, high polymorphism, genome-wide distribution, small amounts of DNA required for analysis, and repeatability of results (Jiang 2013a, b). Quick extraction and detection procedures are required to provide timely information for breeding. Close linkage with the target gene or genes is necessary. Especially when more than one trait is simultaneously undergoing marker-assisted selection, efficient data processing, retrieval, and analysis can become necessary.

As compared with classical breeding, molecular or marker-assisted breeding requires capital investment in sophisticated equipment and infrastructure to house and maintain this equipment, investment in constant equipment modernization or its replacement for maintaining or increasing efficiency, expendables, and dedicated labor coordinated with the breeder. This amount of capital is often not within the reach of public breeders though multinational companies can and do have the capital and facilities for marker-assisted breeding. The knowledge gained through private enquiry into mapping, however, is not shared publicly.

For the five cosmopolitan cucurbit crops, draft genomes and re-sequenced genomes have been published http://cucurbitgenomics.org. They can provide a base for molecular mapping and marker-assisted selection in cucurbit crop breeding.

For watermelon, there has been genome-wide development and characterization of SSR markers, of which over 30,000 were identified (Zhu et al. 2016a, b). The 32 most highly informative SSR loci were used to evaluate the genetic diversity of collections of watermelon germplasm. The thousands of SSRs developed are a large resource for map molecular mapping. A high-resolution genetic map has also been constructed from over 10,000 SNP markers (Reddy et al. 2014).

For melon, the first map with molecular markers, containing isozymes, RFLPs, and RAPDs, was published over 20 years ago (Baudracco-Arnas and Pitrat 1996). Many have been published since then, including using SNPs derived from genotyping-by-sequencing (Chang et al. 2017). An investigation of genome-wide linkage-disequilibrium revealed over 23,000 informative SNPs, a number of which were mapped to narrow intervals overlapping known genes of phenotypic effect, as well as to QTLs affecting fruit shape (Gur et al. 2017).

For cucumber, a number of genes or QTLs have been tagged with molecular markers. The first cucurbit QTLs to be molecularly mapped were in cucumber in 1995 and over the following decade cucumber QTLs for earliness and fruit size and shape were mapped (Wang et al. 2006). A new genetic map for cucumber shows 61 genes, including 10 QTLs, in all seven linkage groups (Weng and Wehner 2016–2017). Using new genomic technologies, chromosome locations and diagnostic markers were reported for 81 simply inherited traits and major-effect QTLs, as well as several hundred QTLs for 42 quantitatively inherited traits (Wang et al. 2020a, b).

For pumpkin and squash, mapping has lagged behind the other three leading cucurbit crops (Wang et al. 2006). Nonetheless, over 100,000 SNPs have been detected and several thousand have been located and mapped (Montero-Pau et al. 2017).

The draft genomes and resequenced genomes of the five leading cucurbit crops have greatly accelerated mapping efforts (Grumet et al. 2017). Molecular mapping of cucurbits is advancing through the utilization of ever faster and more efficient genomic technologies.

3. Breeding objectives

Most cucurbits are produced for their fruits. The major objectives in breeding for cucurbits are for high yields of high quality produce. Both of these objectives contain many components and are impacted by the environment of biotic and abiotic factors.

Breeding objectives are determined in large part by requirements associated with cultivar-groups (morphotypes) and market types. Pumpkin and squash of *Cucurbita pepo* and melons, *Cucumis melo*, are extremely diverse and have been formally classed into cultivar-groups (Paris 1986; Pitrat et al. 2000). Cultivar-groups are designated on the basis of fruit shape, size, color, and other characteristics and accessions within the same cultivar-group are more closely related to one another than they are to those of other groups (see for example, Gong et al. 2012). Cultivar-groups can contain more than one market type. Market types often differ among production areas and across political boundaries, and market demands for a particular fruit type often change over time.

Breeding of cucurbits has been conducted since prehistoric times. For pumpkin and squash, *Cucurbita pepo* and *C. moschata*, evidence indicates that they have been cultivated for at least 10,000 years (Paris 2016a, b). Watermelons, *Citrullus lanatus*, and melons, *Cucumis melo*, have been cultivated for perhaps 5000 years and cucumber, *Cucumis sativus*, for perhaps 4000 years. Wild cucurbits have bitter fruits, though some wild plants bearing non-bitter, bland fruits can occur occasionally. For all of the cucurbits, it is quite likely that the first trait that people selected for was lack of fruit bitterness. Selection for larger fruits likely followed soon thereafter. Subsequently, plants were selected for improved palatability of the fruits, be it sweetness, moistness, and lack of tough fiber. Some of this breeding progress is recorded in the literature and iconography of antiquity, and in archaeological findings. Sweet watermelons were developed by the second century in the Mediterranean region and sweet melons by the tenth century in Central Asia. Pumpkins, winter squash, and summer squash were raised in the Americas and cucumbers were grown in southern Asia. With the Columbian exchange from 1492, these five crops expanded under cultivation to many regions of the world. Prior to 1900, many cultivars of watermelon, melon, pumpkin, squash, and cucumber had been bred, both in their ancestral lands and in their adopted lands, and the cultivar-groups of *Cucurbita pepo* and *Cucumis melo* were already established.

Since 1900, with the advent of knowledge of genetics, breeding of cucurbits has intensified and accelerated. Among the most universally important traits are yield, quality, earliness, storage life, and tolerance to biotic and abiotic stresses. The traits most emphasized differ regionally, some being dictated by limitations of climate and others by consumer preference or market type. The goals of breeding cucurbits are manifold and the lack of one key trait or trait component can prevent a cultivar from becoming a widespread commercial success (Wehner 2008). Breeders usually start with a successful, leading cultivar and making crosses between it and leading cultivars from other areas, or accessions excelling for one or more important traits.

For watermelons, important traits include a high level of fruit-flesh sweetness and intense fruit-flesh color, with red being preferred in most regions, yellow or orange in a few others or in niche markets (Wehner 2008). The flesh should have at least 10% soluble solids as measured with a refractometer, though the goal should be to reach the 14% frequently encountered in the most elite cultivars. The fruit flesh should have “real watermelon flavor”, not the off-flavor found in some obsolete cultivars, a trait which is independent of soluble solids content. The texture of the fruit flesh preferred by most can be described as wet, finely grainy and not fibrous, and firm but not hard. The seeds in the fruit flesh should be small and not excessive in number. External appearance is important, too. Around the world, most consumers prefer fruits that are boldly striped and, in most areas, that the darker stripes are broader than the lighter ones. For durability in long-distance shipping, the rind needs to be tough and of a thickness appropriate to the size of the fruit. Fruit size is also important. In the past, it would seem, the bigger the better with at least 11 kg desired. Growers preferred them because of higher yields and less fruit to harvest per unit area, but with small family units increasing in frequency in many countries, medium and smaller fruit sizes, less than 11 kg, are preferred. Fruit shape in some areas may have some consumer importance, usually blocky oval being preferred. Earliness of fruit ripening is an important consideration in cool, short-season areas or for being first on the market in warm regions. Growers much prefer high-yielding cultivars with intermediate vine length for easier management of the field. In the 1960s, the first triploid, seedless watermelons were introduced. Today, seedless watermelons are grown in many areas. As the pollen of triploid watermelons is sterile, normal diploid plants have to be interspersed in the field for pollination and fruit development. Growers would prefer more efficient pollen producers to ensure maximum yield of triploid watermelons. Finally, growers prefer cultivars carrying resistance to diseases, pests, and abiotic stress-induced disorders, as an insurance policy to obtain good yield and quality.

For melons, important traits across all cultivar-groups would be thick and firm fruit flesh with a small seed cavity (Pitrat 2008). Of the melons used when mature, the flesh should be sweet, having at least 9% soluble solids. Fruit-flesh color options are not as wide in melons as in watermelons, the colors being light green, orange, cream, or white, the preferred color most often orange but highly dependent on the market. The flesh should be mostly juicy but not wet, smooth and not at all fibrous, and moderately soft to firm, depending on the cultivar-group. Most importantly, the flesh should not develop off flavors at ripening and for as long as possible after ripening. Likewise, the mature fruit should be firm and soften somewhat after ripening. External appearance varies widely in melons and is highly dependent on cultivar-group and market type. Casaba melons, in general, are expected to be wrinkled with no to slight or moderate netting whilst muskmelons produced in the western U.S.A. are expected to be scarfaces, completely covered with thick, ropy netting. In most markets, fruit size is acceptable at 1–3 kg. As with watermelons, growers prefer earliness of fruit ripening in cool, short-season areas or for being first on the market in warm regions. Unlike watermelons, shelf-life is an extremely important consideration in melons, as the shelf-life of fruits from a number of cultivar-groups has, traditionally, been limited to several days. Growers prefer cultivars carrying resistance to powdery mildew and other diseases, pests, and abiotic stress-induced disorders. The melon cultivar-groups grown for immature fruits need to have quality traits similar to cucumber (see below).

For pumpkins and winter squash, like melons, important traits are thick and firm fruit flesh with a small seed cavity (Loy 2004; Ferriol and Pico 2008). Pumpkins and winter squash come in a huge variety of sizes, shapes, and color patterns in shades of green, orange, and yellow. Unlike watermelons and melons, pumpkins and squash accumulate starch, as well as sugar, in the maturing fruit flesh, which is too firm to be eaten raw, becoming palatable after cooking. High total dry matter, starch, and sugar contents are good positive indicators of fruit-flesh quality. Peak accumulation of dry matter, mostly starch, in the fruit flesh occurs around 30–40 days past anthesis, the fruit flesh attaining 20–30% dry matter in the highest quality cultivars. Some of the starch is then gradually converted to sugars, and approximately 10–15 days later complete ripeness and peak sugar accumulation occur. Soluble solids content, measured with a refractometer after freezing and thawing the fruit flesh, needs to be 10% for good quality, in the best cultivars averaging 13% and even achieving 20% or more. Unlike watermelons and melons, pumpkins and winter squash can become sweeter during the first few weeks of storage, because the starch is converted to sugar. A fine "melting" texture is an important component of fruit quality, and fruits too low in starch content have a texture that is too thin, fibrous, and moist. Fruit-flesh color is also an important component of quality. Fruit flesh with intense color has more carotenoids, which are antioxidant compounds. Orange is preferred over yellow, and fruits having pale-colored flesh can be unsaleable. The fruits should have non-lignified rinds, for easy slicing during culinary preparation. Pumpkin and squash growers prefer early-bearing cultivars. More and more, growers also prefer cultivars having semi-bush growth habit, which bear earlier and are easier to manage in the field than cultivars having the traditional viney growth habit. Cultivars having a relatively long shelf-life are preferred as well, and growers also value cultivars that have some tolerance to the diseases prevalent in their area. In pumpkins and winter squash, there is a trade-off between dry matter content of the fruit and productivity. Large-fruited cultivars and those that have high yields per unit area tend to accumulate less dry matter in the fruit flesh, due to inter-fruit competition for an inadequate supply of photosynthates.

For summer squash, like melons, pumpkins, and winter squash, important traits are thick, firm fruit-flesh with a small seed cavity (Paris 2008). On the other hand, unlike melons, watermelons, pumpkins, and winter squash, which are mature, ripe fruits four weeks or more after pollination, many of the other desired traits of summer squash differ because they are young fruits, less than a week after pollination. Summer squash are too young to have developed much dry matter, sugars, or other secondary metabolites. Fruit shape and color are extremely important consumer preferences, and the preferences strongly differ among regions and countries. Crooknecks, yellow in color, are preferred in the southeastern U.S.A. and straightnecks and zucchini in the north; vegetable marrows are preferred in the Middle East and cocozelle in much of Italy; scallops (patissons) and zucchini in Australia; worldwide, the zucchini is by far the most popular. Fruit gloss is an indicator of freshness and fruits that grow more quickly are glossier. Earliness and yield are extremely important to summer squash growers. Bush growth habit, erect in stature and open in structure, is important to growers for ease and quickness of harvest, and lack of prickles on the petioles for less fruit damage. The shelf-life of summer squash is extremely short if not packaged or refrigerated, often just one or two days, so longer shelf-life cultivars are preferred as well as those that are less susceptible to chilling injury. Growers also prefer cultivars that are resistant to viral diseases, which can be highly destructive to summer squash crops.

For cucumber, like the other cucurbits, important traits are thick, firm fruit flesh and a small seed cavity (Staub et al. 2008). As cucumbers are used when immature, the other traits of importance are more like those of summer squash than watermelons, melons, pumpkins, or winter squash. Cucumber production is a highly specialized industry as is the industry of processing of cucumbers. Some cucumbers are grown for fresh market, others for pickling. Some for fresh market are grown under protected conditions, some in the open field. Consumers in various regions and countries have their own expectations concerning appearance of the fresh or brined product, whether the fruits should be relatively long or short, and whether they should be coarsely warted or with numerous small warts or non-warted, and whether they should be thin-skinned or if there is no preference. Whatever the case, earliness and yield are of utmost importance to growers, and the deployment of gynoecy in horticulturally acceptable germplasm beginning in the 1960s was a major boost to cucumber growing. Modern greenhouse-grown cucumbers are multiple-flowering gynoecious and parthenocarpic, setting fruits without pollination. The fruits of some cucumber cultivars have slight residual bitterness of the fruits that is expressed under some conditions, and the lack of this tendency toward bitterness is highly desired by growers and consumers. Cucumber cultivars intended for processing need to lack postharvest disorders of the fruit-flesh and seed cavity. Cultivars carrying resistance to the fungal diseases downy and powdery mildew are desired by many cucumber growers.

4. Classical breeding achievements (yield, quality, stress resistance)

Cucurbits have come a long way from being the small, seedy, bitter-fruited fruits of the wild to being the large, succulent, enjoyable fruits that they are in modern cultivation. There are differences as well as parallels among the cultivated species of Cucurbitaceae in the diversity that each exhibits today. The most salient parallels are large, non-bitter, tasty fruits. The differences are the product of both, the ancestral gene pool and consumer-driven selection that cucurbits have been undergoing over many centuries.

The achievements of classical breeding of cucurbits in the 120 years since the rediscovery of Mendelian genetics have been stunning. Efficient progress in breeding was made by this knowledge combined with the development and implementation of controlled pollination procedures for cucurbits (Staub et al. 2008). In the mid-twentieth century, heterosis of cucurbits was harnessed, with seeds of the first commercial F_1 hybrid cucurbit cultivars being marketed. The marketing of heterotic F_1s was an enormous boon to cucurbit crop production. Techniques were developed to make F_1 seed production less labor intensive and thereby less expensive, and marketable to growers for a reasonable price. The popularity and immense production worldwide of the five leading cucurbit crops owes a lot to these achievements.

Watermelon breeding has achieved much since the rediscovery of Mendelian genetics in 1900 (Wehner 2008). Development in the twentieth century of cultivars with tough, flexible rinds has revolutionized the watermelon-growing industry by allowing for long-distance shipping. Resistance to fusarium wilt was introgressed into dessert watermelons from citron watermelons in the early twentieth century and is carried by many extant cultivars. Cultivars have been developed that produce fruits with fewer and smaller seeds. Also developed were cultivars having more thrifty foliage or having earlier fruit ripening. Cultivars having improved external appearance and fruit-flesh color, texture, taste, and soluble solids content have been developed, perhaps the most outstanding of these being ‘Crimson Sweet’ and ‘Allsweet’. The crowning achievement of watermelon breeding over the past 120 years has been the development of seedless cultivars. First conceived by Japanese scientists in the 1930s, seedless watermelons are triploids derived from crossing artificially induced tetraploids, as female parents, with normal, seeded diploids, as male parents. Seedless watermelons first became a commercial item in the 1960s, in the United States. Difficulties in seed production and germination stymied their production until the 1980s, but since then seedless watermelons have replaced seeded watermelons in many markets of economically advanced countries.

Major achievements in melon breeding include the introgression of resistance to diseases, especially powdery mildew, from exotic, non-sweet melons into advanced sweet-melon germplasm (Pitrat 2008). Powdery mildew is a real crop spoiler, attacking the plants when the fruits are well-developed, by reducing foliage area and thus the quality and sweetness of the fruits. ‘PMR 45’ was developed for the melon-growing industry in southern California and released in the 1930s. Another important achievement was the introgression of resistance the soil-borne fungus *Fusarium*, which causes wilt of entire plants. Melons having sweet flesh, including the muskmelons, cantaloupes, and casabas, have undergone much improvement for fruit quality and shelf-life due to classical breeding over the past 120 years.

Major achievements in pumpkin and squash breeding include, first and foremost, breeding for improved fruit quality (Ferriol and Pico 2008; Paris 2018). *Cucurbita maxima* ‘Buttercup’, bred in North Dakota, U.S.A. and released in the 1930s, has since its release been the standard for quality in pumpkins and squash. The high-quality kabocha-type squash, which was developed in part from ‘Buttercup’, is an established crop and industry in lands of the western Pacific. *C. moschata* ‘Butternut’, also introduced in the U.S.A. in the 1930s, is a small, early-bearing, high quality squash that has become a familiar market type in various countries. *C. pepo* ‘Winter Luxury’ is a high-quality pumpkin developed in the southwestern U.S.A. early in the early twentieth century. High-quality acorn squash were also developed in the U.S.A., and high-quality *C. moschata* tropical pumpkins there and in Puerto Rico. A notable achievement for maintaining dependably high fruit quality was the introgression of resistance to powdery mildew from the wild species, *C. okeechobeensis* (Small) Bailey. Another major achievement has been the introgression of bush or semi-bush growth habit, replacing vine growth habit, in high-quality germplasm. This change in plant architecture has made for increases in yield and provided increased cover for developing fruits. Other achievements have included refinements of fruit shape and color.

Major achievements in breeding summer squash, *Cucurbita pepo*, include increased yield in each of the six cultivar-groups of this crop (Paris 1996). Increased yield has been achieved by several factors, foremost plant sexuality. Widely grown, modern commercial hybrids produce a higher proportion of pistillate flowers, and thus they yield more and begin to bear fruit earlier than their predecessors. Modern cultivars have open growth habit, which is a prerequisite for efficient, less laborious harvesting of the fruits. They are also less prickly, for less scratching of the fruits when picked. Many of the newest hybrids also carry resistance to powdery mildew and resistance to one or more viruses, which can wreak havoc with summer squash crops. These resistances have been introgressed by breeders from other species of *Cucurbita.*

Two of the greatest achievements in classical cucumber breeding have been isolating and introgressing gynoecy and parthenocarpy into horticulturally desirable gemrplasm (Staub et al. 2008). These two traits result in much greater yields per unit area. Most field-grown cucumbers today are gynoecious F_1 hybrids, with pollination effected by interspersal of monoecious plants in the otherwise gynoecious population. Most greenhouse-grown cucumbers are gynoecious and parthenocarpic, with no need for pollination. Gynoecious, parthenocarpic plants have exceptionally high yields over an extended period of time. The development of horticulturally desirable gynoecious germplasm was facilitated by developing methodologies for temporary sex conversion of gynoecious plants to monoecious. Another major achievement of cucumber breeding has been the introgression of genes conferring disease resistance into horticulturally desirable germplasm and the development of disease-resistant F_1 hybrids. This was made possible by the development of seedling screening methodologies developed by cooperative efforts of breeders and phytopathologists.

Publicly funded programs into classical breeding and germplasm research into cucurbits has been dwindling over the past 40 years or so. Funds have been siphoned over to more fashionable research in genetic engineering, molecular biology, molecular genetics, and now genomics and other -omics. Apparently, nearly all further accumulated breeding knowledge and experience will become privately owned and therefore secretive. Classical plant breeding is not just based on science, it is also an art and, in spite of its remarkable achievements over the past 120 years, its future under the leadership of scientists in the public domain is in doubt.

5. Limitations of traditional breeding and rationale for molecular breeding

For all of their impressive successes, traditional breeding programs are not always capable of completely achieving their goals or doing so in a timely fashion. Many tools and methodologies have been developed over the years to enhance and quicken breeding. For example, artificially inoculating seedlings with a pathogen to distinguish between resistant and susceptible genotypes. Nonetheless, there are many traits which are complex and quantitatively, polygenically inherited, including the supremely important yield and quality, and abiotic stress tolerance, each of which have many components. Selection for improved yield, quality, and stress tolerance, while heretofore largely successful, could become more quick and efficient if knowledge of their components and methodologies to analyze these components are developed. Herein lies the rationale for molecular breeding.

Molecular breeding can be defined as the application of molecular tools in breeding (Jiang 2013a, b). The most widely applied form of molecular tool in plant breeding is DNA markers from loci closely linked to loci affecting important phenotypic traits. Presently, the most commonly used markers are simple sequence repeats (SSRs), also known as microsatellites, and single nucleotide polymorphisms (SNPs). The linked markers serve breeders by allowing them to select individuals of a large population for phenotypic traits based on the presence or absence of markers instead of having to grow out the populations in the field.

DNA markers are especially potentially useful in attempting to breed for improvement of quantitatively inherited traits such as yield, quality, and abiotic stress tolerance. The many genes affecting quantitative traits are known as quantitative trait loci (QTLs). Successful application of QTLs in breeding depends on the magnitude of their effects and their expressivity across various genetic backgrounds and a wide range of environments. Selecting individuals in a population for particular QTLs on the basis of phenotype only is difficult. However, selection for QTLs can be assisted by the use of DNA markers.

Large numbers of molecular markers and high density genetic maps are available for many crops (Moose and Mumm 2008). QTLs can be found to associate, positively or negatively, with particular molecular markers. Moreover, mapping has provided estimates of the number of loci and allelic effects of particular quantitative traits. Fine mapping can ascertain the positions of QTLs in the genome, thereby facilitating the testing of their presumed effects across breeding populations. Identification of tightly linked markers allows the breeder to use them in both, backcross breeding and pedigree breeding or recurrent selection.

The primary use of molecular markers has been as DNA “fingerprints”. They have been widely applied for studying relationships among genera, species, and infraspecific taxa, including among cultivars and the myriad of accessions used for breeding. As germplasm fingerprints, they have had important uses in identification of proprietary genotypes, serving as hard evidence in legal proceedings. Though they can be a potentially useful tool for plant breeding programs to help make selection more efficient, they are not without limitations.

Like the many heralded and well-funded technological advances that siphoned academic and public support from classical plant breeding, molecular mapping can be a helpful tool for plant breeding. It has the potential to be especially helpful for obtaining an improved perspective of the crop genome and gene pool, for introgressing genes for biotic and abiotic stress resistances from wild crop relatives, and for pyramiding genes for resistance or other traits. Molecular marker technology and marker-assisted selection have long been promoted as expediters of transfer of desirable genes among breeding lines, of genes from wild relatives to crop plants, and facilitate analysis of individual genes in quantitative traits (Tanksley et al. 1989). However, the facts on the ground, years later, are much more modest. While breeding using molecular markers or marker-assisted selection has been effective for alleles having large phenotypic effects, it has not been, for the first 20 years at least, successful in breeding for quantitatively inherited traits such as yield, quality, and abiotic and biotic stress tolerance (Bernardo 2008; Jannink et al. 2010). Rather, the research into the development and application of molecular markers in plant breeding was funded by donors with a “strategic science quality” or biotech-oriented advocacy, resulting in much funding and many publications in scientific, peer-reviewed journals, in which the everyday technicalities of translating the results into benefit for practical plant breeding were not addressed (Xu and Crouch 2008).

The major cause for concern among plant breeders, especially among those breeding crops for organic, sustainable, and low-income agriculture, has been the large gap between genotyping and phenotyping, especially for quantitatively inherited traits of complex or unknown inheritance, such as nutrient-use efficiency, and about the cost of accessing efficient marker technology (Lammerts van Bueren et al. 2010). Enhanced cooperation between practitioners of classical breeding with those possessing skills in molecular genetics would be beneficial but it may not solve all or most of the problems encountered in attempting to bridge the genotype—phenotype gap. Among classical plant breeders, too, there are doubts that molecular markers will soon be available for more complex traits that are major goals of practical breeding, especially in organic or sustainable agriculture, and that phenotypic selection in the field was still the best approach. Individual protocols for comparisons of results in the field and laboratory would have to be devised for each trait targeted for breeding.

Given the limitations of marker-assisted selection, a more recent proposal has been to apply genomic selection to plant breeding (Jannink et al. 2010). Instead of searching for individual loci associated with a desirable trait, genomic selection “uses all marker data as predictors of performance”. Selections from a segregating, breeding population would be based on genomic-selection predictions. Genomic selection was recently deployed in an attempt to breed for resistance to a common insect pest of summer squash, *Cucurbita pepo.* However, this approach failed to achieve a noticeable gain in the field, and the authors concluded that better strategies would likely entail leveraging efficiencies from phenotyping-associated traits and methods for shortening breeding cycles (Brzozowski and Mazourek 2020). On the other hand, genomic selection was successfully deployed in breeding for improved quality of butternut squash, *C. moschata* (Hernandez et al. 2020).

A new approach to molecular mapping is genome-wide association analysis. Using this approach, it has been possible to map qualitative traits to narrow genomic intervals overlapping with known causative genes as well as map QTLs, for example, in melon (Gur et al. 2017).

In 2009, the cucumber genome was sequenced (Huang et al. 2009). Since then, the genomes of watermelon, melon, pumpkin, squash, and cucumber have been sequenced and resequenced using high-throughput technologies (Casacuberta et al. 2017; Weng 2017; Guo et al. 2019; Wu et al. 2019; Xanthopoulou et al. 2019; Cucurbit Genomics Database 2020). Many polymorphic, codominant, and transferable SSR and SNP markers have been discovered by sequencing. These are expected to serve as the basis for high-density genetic linkage maps and gene identification (in pumpkin, for example, see Nguyen et al. 2020) as well as use them for marker-assisted selection (Fukino and Kawazu 2016). Knowledge of genome sequences of cucurbit taxa serves as a basis for performing comparative analyses among them (Grumet et al. 2017; Zheng et al. 2018). Synteny across cucurbit genomes and genomic regions lacking synteny, that is, taxon-unique sequences, may facilitate gene identification and homology across taxa as well as stimulate the imagination of cucurbit breeders to new traits and trait combinations for the various cucurbit crops.

6.3 Brief on Diversity Analysis

Cucumber

Cucumber (*C. sativus* var. *sativus)* is occupied by low levels of genetic diversity, especially in contrast with other cross-fertilized species of the Cucurbitaceae, such as melon (Esteras et al. 2011). With the aim of preserving the valuable genetic material of varieties and the valid differentiation of closely related varieties, Staub et al. (2005) tried to develop a reference marker array (using 155 SSRs and Sequence Characterized Amplified Region (SCARs) markers). According to their findings, the mission of discrimination of this genetic material proved to be quite difficult and therefore other types of markers (such as SNPs) seems to be more suitable for assessing the diversity of these cultivars. Pandey et al. (2018) studied the genetic diversity of 40 cucumber genotypes representing 12 major states of India and covering 6 agro-climatic zones analyzing their morpho-physiological traits compared with 12 EST-SSRs markers. They revealed a low genetic diversity among the Indian genotypes studied, in contrast to high diversity levels of morpho-physiological traits.

Recent evolution in plant genomics contributes to a better understanding and more thorough study of genetic diversity at molecular level. DNA markers is a common method of studying the genetic diversity in cucumber, used by many researchers (Pandey et al. 2008; Sikdar et al. 2010). Among these markers genomic SSR (gSSR) markers are not so informative due to their small correspondence with transcribed sequences. On the contrary, EST-SSRs markers can be more informative and therefore can be used in functional diversity studies, especially in gene rich regions of the genome (Zhang et al. 2005). Furthermore, EST-microsatellite (eSSR) polymorphism is linked with transcribed sequences of the genome and represents the genetic diversity of genes (Varshney et al. 2005). EST-SSRs are generally created by EST databases (Xanthopoulou et al. 2017). In order to map traits of interest and DNA fingerprinting, EST–SSRs have been used in watermelon (Hwang et al. 2011) cucumber, pumkin (Xanthopoulou et al. 2017) and melon (Levi et al. 2006a, b; Kong et al. 2007).

Melon

Melon is the model plant of Cucurbits for genetic and genomic studies. Many studies aimed to the generation of sequence databases (González et al. 2010, Gonzalez-Ibeas et al. 2007, http://www.melogen.upv.es; www.icugi.org) and one of their main tools is single nucleotide polymorphism (SNP) detection. Morales et al. (2004) with the use of ESTs, was the first team that identified SNPs in melon. They reported an average frequency of 1 SNPs per 441 bp between two *inodorus* genotypes. In order to advance their study one step further, they detected 356 high-quality SNPs after screening 30,000 ESTs sequences from four genotypes of melon (González-Ibeas et al. 2007). Deleu et al. (2009), studied the genetic diversity in various melon accessions and reported the genetic relationships and the results were corresponding to those of the study of SSRs markers.

Molecular markers have been applied in order to discriminate and identify melon genotypes from USA and Europe (commercial cultivars, hybrids and breeding lines) mainly classified to *cantalupensis* (Charentais, Shipper, Galia and Ogen) and *inodorus* (Honey dew type and Cassaba Rochet, Piel de Sapo and Yellow Canari types) types. Although market cultivars were separated satisfactorily with the use of molecular markers, their classification was to some extent insufficient due to the introgressions occurred during breeding (Esteras et al. 2011).

Furthermore, decreased levels of genetic diversity have also been observed in some groups of varieties (Garcia et al. 1998; Staub et al. 2000). When wild germplasm from these and other botanic groups were included in the study, the level of genetic diversity was increased. In conclusion, most of the studies that have been conducted, divide the genetic material of the melon into two main groups (ssp. *melo* and ssp. *agrestis*) (Monforte et al. 2003; Nakata et al. 2005; Gonzalo et al. 2019).

The highest percentages of molecular diversity (number of alleles and polymorphic *loci*) were observed in Central Africa and India in contrast to Mediterranean area and China Sea which are the extremes of melon distribution. Stepansky et al. (1999) studied genetic diversity of most of the botanical groups. They used RAPDs in combination to ISSRs markers in order to acquire phenotypic and molecular information from wild, feral and cultivar accessions from 23 countries which represent the primary and secondary centers of diversity (Africa, southern and western Asia and the Far East). Phenotyping focused mainly on traits that enable classification and separation of different botanical groups such as: seed size, stem thickness, pubescence, sex type, ovary shape, ovary pubescence, fruit shape and size, skin color, texture and design, splitting, abscission, external aroma, flesh color, taste, sucrose, glucose and fructose and pH. The subclassification of the groups of varieties was consistent with the classical classification, which proves that the characteristics used for the classical classification are consistent and informative. The molecular data dendrogram was consistent with the phenotypic traits dendrogram. The sweet-fruited *cantalupensis* and *inodorus* grouped together, although they differentiate in ripening behaviour, and the nonsweet varieties *agrestis*, *conomon* and *momordica* clustered together too. The classification of certain botanical groups may differ from study to study (Esteras et al 2011). For example, *dudaim* and *chito* cultivars often are clustered in *agrestis* group, while they have been identified to belong to ssp. *melo*. Moreover, the *flexuosus* types based on the phenotypic characteristics are classified in the group of non-sweet types while based on the molecular data are classified in the group of sweet genotypes. Esteras et al. (2009) analyzed 212 accessions of melon, representing all the genetic diversity of the species, using AFLPs markers and reported that *dudaim* and *chito* types were classified between ssp. *melo* and *agrestis* and *momordica* whereas *flexuosus* types were clustered among both groups.

SSR markers have been extensively used for the assessment of the genetic diversity of melon genotypes. Tzitzikas et al. (2009) studied the genetic diversity and population structure among traditional Greek and Cypriot melon genotypes using 17 SSR markers. Escribano et al. (2012) analyzed the correlation between genetic relationships of Spanish Inodorus landraces with other commercial varieties using 52 SSR markers. Kaçar et al. (2012) used 20 SSR markers in order to study molecular diversity of local Turkish melon accessions. Zhu et al. (2016a, b) designed 42 highly informative SSR markers in order to evaluate the genetic diversity of 118 melon genotypes (14 of subsp. *agrestis* and 104 of subsp. *melo*).

Kishor et al. 2020 used Genotyping-by-sequencing (GBS) and employed genome-wide highly efficient SNP markers with the aim of genetic differentiation of melon cultivars. Furthermore, 9018 SNPs were identified by analyzing 48 commercial Korean melon cultivars released by seed companies in South Korea. A total of 192 SNPs were detected as putative SNPs.

Watermelon

Watermelons available in the market are varied in many traits, such as: fruit shape, fruit size, flesh color, rind thickness, the amount of seeds and much more. Recently, there has been a decrease in genetic diversity of the genetic resources of watermelon, which is responsible for the reduced evolution in research and breeding. The possible reasons for this reduction are the cultivation practices as well as the selection of varieties with desirable characteristics. The connection of morphological characteristics with the genetic information is of paramount importance in order to obtain comprehensive understanding on breeding and conservation of watermelon genetic material. In contrast to research on the morphological characteristics of watermelon, which is abundant in the international literature (Gbotto et al. 2016; Singh et al. 2017; Pandey et al. 2019), research on genetic diversity is incomplete.

C. lanatus var. *citroides* (citron watermelon) and *C. lanatus* var. *lanatus* (dessert watermelon) are the most popular species of watermelon (Mashilo et al. 2017). Dessert watermelon is common for its narrow genetic base, unlike citron melon which is known for its high genetic diversity (Pandey et al. 2019; Ocal et al. 2014). In order to estimate genetic diversity of watermelon, various molecular markers have been used. With the aim of evaluation of genetic relationship among cultivated watermelons and *Citrullus* species various molecular markers have been used (Lee et al. 2019). These results reported low DNA polymorphism among cultivated watermelons in contrast to *Citrullus* subspecies which have high levels of genetic diversity (Zhang et al. 2012).

Moreover, Levi et al. (2013), studied cooking citron watermelon and green-white-fleshed types. The green-white fleshed types of watermelon have small fruits (≤10 cm in length), thin rinds, mostly dark brown or black seeds and low diversity in seed coat color, fruit shape and rind stripe. Watermelon fruit size is a feature of great interest to consumers who tend to prefer small size (Gusmini and Wehner 2007). Therefore, small-sized citron watermelons is an excellent source of genetic material for breeding in order to develop new small-sized sweet dessert watermelon cultivars. The orange- and yellow-fleshed citron types have large fruits, thick rinds and possesses high phenotypic diversity in fruit shape, color, rind stripe patterns and seed coat color. In the future it would be interesting to study whether these phenotypic differences reflect changes in the genetic profile.

In watermelon, various SSRs and EST-SSRs markers have been revealed and used for genetic diversity evaluation (Jarret et al. 1997; Guerra-Sanz 2002; Joobeur et al. 2006; Verma and Arya 2008). Jarret et al. (1997) distinguished *C. lanatus* var. lanatus from wild and cultivated citron and *C. colocynthis*. Citron is the putative wild progenitor of var. lanatus and var. citroides which is proved by the many common alleles they have. Joobeur et al. (2006) revealed genomic SSRs from a watermelon BAC library and 95% was polymorphic. Verma and Arya (2008) applied these EST-SSRs in seven Indian genotypes reporting 22% polymorphism and revealing high transferability to Cucurbitaceae genotypes. Jun-Lee et al. 2019 used GBS and SNPs in order to analyze the genetic diversity of two groups of watermelons (1) 41 watermelon accessions at the National Agrobiodiversity Center (NAC) at the Rural Development Administration in South Korea and (2) 27 Korean commercial watermelons. They reported four different groups within the populations.

The genus *Citrullus* consists of seven species (Chomicki et al. 2015). *Citrullus naudinianus* originated from sub-Saharan Africa, is the only diecious and most morphologically distinctive species (Grumet et al. 2017). *Citrullus ecirrhosus* and *Citrullus rehmii* originated from southern Africa, grow better to desert environments. *Citrullus colocynthis* is found in northern Africa and southwestern and central Asia (Paris 2015) and it is cultivated for its seed oil and medical application. The wild form of *Citrullus amarus* is endemic in southern Africa and it is cultivated throughout the Mediterranean region for numerous uses such as jam, animal fodder and as a source of water (Renner et al. 2017). *Citrullus mucosospermus* is found western Africa and it is cultivated for seed consumption (Renner et al. 2017). *C. colocynthis*, *C. amarus* and *C. mucosospermus* have been used in order to evolve breeding programs of sweet watermelon as to disease and pest resistance (Grumet et al. 2017).

Pumpkin and Squash

Cucurbita pepo is the species with the greatest variability in fruit traits (Naudin 1856). After Columbus arrival to America, the range of variability of *Cucurbita* spp. has increased in America, Europe and Asia (Decker 1988). Eight distinct morphotypes based fruit shape constitute the classification of edible cultivated types of this species (Paris 1986; Paris 2001): pumpkin, vegetable marrow, cocozelle and zucchini (ssp. *pepo*), scallop, acorn, crookneck and straightneck (ssp. *ovifera*). Most of them are grouped as summer squashes, except for pumpkin and acorn. The subspecies *pepo* consists of ornamental types with orange, round and smooth fruits unlike ssp. *ovifera* var. *ovifera* which consists of ornamental types with oviform and pyriform fruits. ssp. *pepo* is possessed by greater variability in morphological traits of agronomic interest (fruit and seed quality traits, pest and disease resistance) (Paris 1998, 2001a; Paris and Nerson 1998; Darrudi et al. 2018).

Apart from morphological variation, molecular diversity within the species evaluation was conducted with the use of different marker systems. In order to assess the genetic variability within *C. pepo*, various markers have been used (allozymes, restriction RFLPs, RAPDs, AFLPs and ISSRs) (Lebeda et al. 2006; Esteras et al. 2011). Research to date has focused on the study of evolution and genetic relationships between *C. pepo* subspecies, between the cultivated genotypes and between the domesticated and their wild relatives (Ferriol et al. 2003; Paris et al. 2003).

Xanthopoulou et al. (2014) studied the genetic diversity of thirty six summer squash landraces from Greece with the use of start codon targeted (SCoT) polymorphism and inter-simple sequence repeat (ISSR) markers. They conducted informativeness and efficiency analysis of SCoT and ISSR molecular markers and they reported that ISSR markers were more polymorphic (PIC value = 0.237) and the highest marker index (1.503) was found to SCoT markers. Moreover, with the aim of 36 summer squash landraces originated from Greece genotyping, Xanthopoulou et al. (2013) applied high-resolution-melting (HRM) analysis method combined with expressed sequence tags–simple sequence repeat (EST–SSR) markers. The results showed that the EST–SSR markers used were informative producing a unique melting curve for each genotype. Therefore, they are suitable for comparing and distinguishing these genotypes. Furthermore, they suggested that only four microsatellite markers are adequate for the 36 summer squash landraces diversification.

Kaźmińska et al. (2017) analyzed the genetic diversity within 85 *C. maxima* genotypes with the use of 23 highly informative SSRs. This collection was composed of cultivars and breeding lines from Europe, North America, Asia, Australia, and New Zealand.

Moon et al. (2019), with the use of 39 SSR markers, analyzed genetic relationships between resistant and between susceptible genotypes of subspecies *pepo* (Zucchini and Pumpkin) and *texana* (Acorn, Straightneck, and Crookneck). They reported that resistant morphotypes of subspecies *pepo* are genetically closer than those of subspecies *texana.*

Paris et al. (2003) and Ferriol et al. (2003) studied thoroughly *C. pepo* species. More specifically, Paris et al. (2003) analyzed *C. pepo* genotypes from three subspecies with AFLPs, ISSRs and SSRs markers, revealing a high correlation between them. Furthermore, they reported higher levels of variation between domesticated groups of genotypes than between wild groups, which contradicts the results of Decker-Walters et al. (2002b), that revealed higher variation in wild populations. Various studies with allozymes (Ignart and Weeden 1984), molecular markers (Torres Ruiz and Hemleben 1991; Katzir et al. 2000) as well as botanical classification studies had similar results. Moreover, the classification within ssp. *ovifera* groups was coherent with morphotypes classification revealing six groups: acorn, crookneck, scallop, straightneck, *ovifera* gourds and wild genotypes. Scallop group, which is the putative oldest group, is genetically close to the *texana* gourds, proving that it might have been derived from ancestors by American natives. Little phenotypic variation was revealed within straightneck, (ssp. *ovifera*) (Paris 2000). Zucchini is the most recent and distinct group in contrast to the other morphotypes of ssp. *pepo* (Paris 2000). The great diversity of cocozelle, vegetable marrow and generally pumpkin accessions makes it difficult to assess the genetic relationships among them.

Ferriol et al. (2003), evaluated the genetic diversity of different morphotypes of Spanish landraces and unclassified genotypes with the use of SRAPs and AFLPs markers. Their results were consistent with those published by Paris et al. (2003). Based on the results of the SRAP markers, it was found that the classification within the ssp. ovifera morphotype was more specific compared to the ssp. pepo morphotype. In the ssp. *ovifera* group, crookneck and straightneck clustered separately from scallop, whereas acorn was more scattered.

C. moschata consists of many landraces with divergent characteristics originated in different regions. Despite the great diversity of *C. moschata* landraces, only a few types end up in the consumer: “Cheese” type, “Crookneck” type and “Bell” type (Whitaker and Davis 1962). “Butternut” was the first commercial cultivar, derived from Crookneck type (Esteras et al. 2011). Numerous publications concerning the study of the morphological and agronomic characteristics of different landraces from different centers of diversity have been conducted. Wessel-Beaver (2000) revealed high frequency of specific traits in Colombian landraces. Labrada et al. (1997)

grouped Cuban landraces according to their morphological traits and yield. Furthermore, several studies analyzed cultivars with bushy growth (Wu et al. 2007). Bushy growth has been observed and studied in *C. pepo* and *C. maxima*, as well as in *C. moschata*, revealing the gene related to the inhibition of cell elongation.

There are numerous publications concerning the study of diversity in quality traits (Esteras et al. 2017 and references therein). Ferriol et al. (2004) assessed morphological and genetic variability of *C. moschata* Spanish landraces and some American genotypes, using AFLPs and SRAPs markers.

Many landraces could not be grouped in any of the commercial clusters. Spanish, Central American and South American accessions were clustered in different groups, revealing some primitive traits. *C. moschata* landraces from the Canary Islands was genetically divergent from those of the peninsula, proving that some genotypes were more adaptable to the tropical climate of the islands (Ferriol and Pico 2008).

Molecular markers and principally DNA markers, are the most validate method for genetic variation analysis in crop species. Simple sequence repeats (SSRs) have been extensively employed for DNA fingerprinting and genetic diversity analysis due to their co-dominant and multi-allelic properties (Bae et al. 2015; Kwon and Choi 2013; Phan et al. 2016; Kong et al. 2020). In order to assess high-throughput genotyping, single nucleotide polymorphisms (SNPs) are the most suitable type of markers. Furthermore the discovery of genome-wide SNPs with the use of Next-generation sequencing (NGS) technologies, make their application more widespread in plant breeding (Hao et al. 2020; Nguyen et al. 2020).

6.4 Association Mapping Studies

Cucumber

The Cucurbitaceae include several economically important cultivated plants, such as cucumber (*C. sativus* L.), melon (*C. melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and squash and pumpkin (*Cucurbita* spp.). The cucurbit family is not only an economically important cultivated plant, but also sets out a rich diversity of sex expression, and the cucumber has served as a primary model system for sex determination studies and plant vascular biology for studies of long-distance signaling events (Lough and Lucas 2006). The greatest achievement in years may be the sequencing of the cucumber genome, for the cucurbit research community. A draft of the *Cucumis sativus* var. *sativus* L. genome sequence was reported recently. The study of Huang et al. establishes that five of the cucumber's seven chromosomes arose from fusions of ten ancestral chromosomes after divergence from *Cucumis melo*. The sequenced cucumber genome gave insight into traits such as its sex expression, disease resistance, biosynthesis of cucurbitacin and 'fresh green' odor. They also identify 686 gene clusters linked to phloem function. The cucumber genome provides a valuable resource for developing elite cultivars and for studying the evolution and function of the plant vascular system (Huang et al. 2009).

Cucumber, *Cucumis sativus* L. a diploid species ($2n = 2x = 14$) provides some advantages for genome research for three reasons, its relatively small genome size (~367 Mbp) (Arumuganathan and Earle 1991), low percentage of repetitive DNA and short life cycle. Since the release of the cucumber genome sequence, significant progress has been made in our understanding of the cucumber genome and to bridge all of this knowledge in order to face the ever-changing needs caused by both the environment and man. This work is a review about the association mapping studies related to abiotic factors, as the plants are often subjected to environmental stresses such as drought, salt, extreme temperature and heavy metals.

The accessibility of draft genome can be used as keyhole to a genome-wide view of the structure, organization and evolution of those significant genes and gene families in the cucumber genome. Many studies have been conducted associated to genome-wide analysis of a number of gene families, especially transcription factor gene families and their response to abiotic stress in cucumber.

The function of *NAC* genes was identified in the cucumber genome and phylogenetically analysed the members of the *NAC* gene family in order to reveal the important roles in plant development, abiotic stress resistance and signalling transduction. The results were the identification of 82 high-confidence *CsNAC* genes were clustered into 13 sub-groups with uneven distribution in the cucumber genome. Additional, *CsNAC* genes were more sensitive to salinity than other stresses and several *CsNAC* genes, including CsNAC35, which is an orthologue of the known stress-responsive Arabidopsis RD26, were identified as highly responsive to abiotic stresses and hormones (Wei et al. 2016).

Forty HD-ZIP (*CsHDZ*) genes identified in the cucumber plant and the phylogeny of the CsHDZ proteins further divides into 4 subfamilies (*HD-ZIP I*, *HD-ZIP II*, *HD-ZIP III*, and *HD-ZIP IV*) based on the structural similarities and functional diversities and the expression under various abiotic stresses and biotic stress implied the potential role of *CsHDZ* genes in mediating the resistance of *Cucumis sativus* (Sharif et al. 2020). The R2R3MYB and bZIP proteins, one of the largest families of transcription factors in plants, playing crucial roles in plant development, physiological processes, and biotic/abiotic stress responses, have identified in cucumber and they demonstrated that 8 out of 55 (14.54%) cucumber R2R3MYB genes subsisted alternative splicing events, producing a variety of transcripts from a single gene, which illustrated the extremely high complexity of transcriptome regulation, specially R2R3MYB transcription factors play important roles in the response to abiotic stresses (NaCl, ABA and low temperature treatments) and 64 bZIP transcription factor-encoding genes in the cucumber genome (Li et al. 2012; Baloglu et al. 2014). In this study, they identified a total of 55 *CsWRKY* genes and the structure of their encoded proteins, their expression, and their evolution were screened. These new WRKY sequences need further investigating the function of *WRKY* genes under various stress conditions (Ling et al. 2011).

In this study, a total of six *CsGPX* genes were identified in *C. sativus* through genome-wide analysis. Their genome distributions, promoter sequences, and expression profiles in different tissues and in response to various abiotic stresses were analyzed. The results of expression profiling of *CsGPX* genes under different abiotic stresses and ABA treatments indicated that one or more *CsGPX* genes might be involved in ABA signaling pathway and stress responses (Zhou et al. 2018). The characterization of cucumber GRAS family was divided into 10 groups according to the classification of Arabidopsis and tomato genes through the screening and the expression patterns of *CsGRAS* genes in different tissues and under cold treatment provides valuable information and candidate genes for improving cucumber tolerance to cold stress (Lu et al. 2020). Moreover, Genome-wide analysis of microRNA targeting impacted by SNPs in cucumber genome indicates that SNPs (19 SNPs in miRNA mature sequences and 113 SNPs in miRNA-target binding sites were identified) can alter miRNA function and produce unique miRNA targets in cultivated and wild cucumbers. Therefore, miRNA-related SNPs may have played important in events that led to the agronomic differences between domestic and wild cucumber (Ling et al. 2017). High-throughput genotyping- by-sequencing (GBS) technology to characterize the United States National Plant Germplasm System (NPGS) collection of cucumber (*Cucumis sativus* L.) was conducted. The GBS data, derived from 1234 cucumber accessions, provided more than 23 K high-quality single-nucleotide polymorphisms (SNPs) that are well distributed at high density in the genome (~1 SNP/10.6 kb). They identified genome regions significantly associated with 13 horticulturally important traits through genome-wide association studies (GWAS), for chilling tolerance, eighteen SNPs were identified, with ten on chromosome 1, one on chromosome 2, two on chromosome 4 and five on chromosome 7 (Wang et al. 2018).

Melon

Melon (*Cucumis melo* L.) belongs to the Cucurbitaceae and it is a diploid plant species ($2n = 2x = 24$) of great interest for its specific biological properties and for its economic importance. The work of Garcia-Mars et al. report the first complete sequence of melon in order to produce high quality draft sequence of melon genome. The melon and cucumber genome sequences are useful tools for giving insights to the genome structure and evolution of two important species of the same genus with different chromosome number (melon, $2n = 2x = 24$; cucumber, $2n = 2x = 14$) (Garcia-Mas et al. 2012). *C. melo* is nominated for breeding and genetic research opportunities, including genome-wide association (GWA) analysis. Due to highly diverse for fruit traits providing wide.

Most of GWAS has been successfully used in melon for identifying the QTLs affecting fruit firmness (Nimmakayala et al. 2016), fruit shape (Gur et al. 2017), soluble solids content (Xu et al. 2013), and other traits (Pavan et al. 2017; Tomason et al. 2013). Genome-wide association analysis (GWAS) applied to explore the QTLs related to chilling tolerance in melon, detecting 51 loci that contributed to 74 marker-trait associations. Of these associations, 35 were detected in the whole panel, 21 in *melo*, and 18 in *agrestis* and nine loci were selected for evaluation of the phenotypic effects related to their alleles, which identified 11 elite alleles contributing to seedling chilling tolerance (Hou et al. 2018).

Glutathione S-transferase (GST) gene family members, which plays a crucial role in plant tolerance to biotic and abiotic stresses, were analyzed from a genome-wide perspective. In this paper forty-nine GSTs were screened in melon genome belonging to eight classes. The results of chromosome mapping revealed that GSTs were present in all chromosomes except for chromosome 5. Gene replication events played an important role in the expansion and evolution of melon GST gene family. The relative expression level of *CmGSTU7*, CmGSTU10, *CmGSTU18*, *CmGSTF2* and *CmGSTL1* in roots of melon seedlings was significantly higher than that in control group, it undermines that the five GSTs might play an important role in cinnamic acid mediated autotoxicity stress in melon (Wang et al. 2020a, b).

Moreover, this is the first report on CDPK and CRK gene families in melon, which provides a basic foundation for functional characterizations of *CmCDPK* and *CmCRK* genes. The identification of the CDPK and CRK gene families, a total of 18 CDPK genes and 7 CDPK-related protein kinases (CRK) genes in the melon and the analysis of their genomic structure and chromosomal distributions and the transcription expression patterns gave new insights into the evolutionary history of the *CmCDPK* and *CmCRK* gene families and reveals a set of potential candidate genes for future genetic modification because plays vital roles in plant growth, development, and responses to multiple stresses (Zhang et al. 2017a, b).

Watermelon

Watermelon (*Citrullus lanatus*) is an important cucurbit crop worldwide. The first high-quality genome sequence of an east Asia watermelon cultivar, 97103 ($2n = 2x = 22$) reported by Guo et al. (2013) and resequencing of 20 watermelon accessions spanning the genetic diversity of *C. lanatus* (Guo et al. 2013). Genome wide characterization of simple sequence repeats in watermelon genome was attempted and total of 39,523 microsatellite loci were identified from the watermelon draft genome with an overall density of 111 SSRs/Mbp, and 32,869 SSR primers were designed with suitable flanking sequences, continued with comparative analysis with two other important crop species in the Cucurbitaceae family: cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.). All this work provides a valuable resource for genetic map construction, QTL exploration, map-based gene cloning and marker-assisted (Zhu et al. 2016a, b). They have conducted many researches related to association mapping studies, trying to afford insight in their application in comparative mapping and genetic diversity analysis.

Genotyping by sequencing reveals large 11,485 SNP markers that in turn generate opportunities in genome-wide association mapping and marker-assisted selection and LD was also detected within individual genes on various chromosomes (Nimmakayala et al. 2014), other project identified a set of 10,480 SNP markers for constructing a high-resolution genetic map of 10,096 cM for watermelon, they also reveal that chromosome 3 consisting of important genes that might have had a role in sweet watermelon domestication (Reddy et al. 2014). Moreover, 32,869 SSR primers were designed with suitable flanking sequences developed from the watermelon genome, providing a valuable resource for genetic map construction, QTL exploration, map-based gene cloning and marker-assisted selection in watermelon which has a very narrow genetic base and extremely low polymorphism among cultivated lines (Zhu et al. 2016a, b).

The genome-wide analysis of the ClLAX, ClPIN and ClABCB family genes, including chromosome localisation, gene structure, and phylogenic relationships, was carried out and 7 *ClLAXs*, 11 *ClPINs* and 15 *ClABCBs* were mapped on 10 watermelon chromosomes, proving the possible roles of auxin transporter genes in watermelon adaptation to environmental stresses under salt, drought, cold treatment and grafting through the Analysis of the expression patterns (Yu et al. 2017). Moreover, genome-wide identification of expansin gene family (Gao et al. 2020), nuclear factor Y (NF-Y) proteins (Yang et al. 2017), NAC transcription Factor Family (Lv et al. 2016), TIFY gene family (Yang et al. 2019) and small heat shock protein 20s (*HSP20s*) (He et al. 2019) have been done, revealing their crucial role in various abiotic stress responses.

Pumpkin

The research in pumpkin and squash is limited about the association mapping. Pumpkin (*Cucurbita moschata*) is an economically worldwide crop, but few quantitative trait loci (QTLs) have been reported due to the lack of genomic and genetic resources, especially for abiotic stress. A high-density linkage map with 3470 SNP markers using ddRAD technology in a population with 200 F2 individuals composed of 20 linkage groups (LGs) and QTL mapping of fruit-related traits performed on carotenoids, sugars, tuberculate fruit, fruit diameter, thickness and chamber width in pumpkin (Zhong et al. 2017). The first work related to cold stress tolerance have been done by Kayum et al., who identified 7 of 32 genes glutathione S-transferase (GST) family (*CmaGSTU3*, *CmaGSTU7*, *CmaGSTU8*, *CmaGSTU9*, *CmaGSTU11*, *CmaGSTU12*, and *CmaGSTU14*), highly expressed in the cold-tolerant line and can be used putative candidates for use in breeding cold-tolerant crop varieties (Kayum et al. 2018).

Future potential for the application of association studies for germplasm enhancement

The narrow genetic base of modern crop cultivars creates a serious obstacle to sustain and improve crop productivity due to rapid vulnerability of genetically uniform cultivars by potentially new biotic and abiotic stresses. Many agriculturally important traits such as productivity and quality, tolerance to environmental stresses, and some of forms of disease resistance are controlled by polygenes that greatly depends on genetic × environmental (G × E) interactions. These complex traits are referred to as quantitative trait loci (QTLs), and it is challenging to identify QTLs based on only traditional phenotypic evaluation. The identification of QTLs of agronomic importance and its utilization in a crop improvement further requires mapping of these QTLs in a genome of crop species using molecular markers. QTL mapping of fruit traits-related traits (carotenoids, sugars, tuberculate fruit, fruit diameter, thickness and chamber width) in pumpkin (Zhong et al. 2017), fruit morphological traits, fruit size characters, and pulp content (Díaz et al. 2017), sugar and carotenoid metabolism genes (Harel-Beja et al. 2010) in melon, fruit size (Weng et al. 2015), fruit flesh thickness (Xu et al. 2015) in cucumbers, flesh quality traits (Fall et al. 2019), fruit shape (Dou et al. 2018), sugar accumulation (Ren et al. 2018), β-carotene (Branham et al. 2017) in watermelon. Consequently, genomic designing for abiotic stress in cucurbits needs to be conducted more researches in order to reveal all the target genes and be mapped using markers. There is only one research have been conducted QTL analysis for low temperature germination (LTG) ability using a recombinant inbred line (RIL) population, providing a useful for marker-assisted selection in breeding for this trait (Yagcioglu et al. 2019).

6.5 Marker-Assisted Breeding for Resistance/Tolerance Traits

Cucurbits are important vegetables cultivated worldwide with high nutritional value. The production of Cucurbitaceae crops has been rising yearly, with increasing consumption demand. Thus, new cultivars that can produce high-quality cucurbits with high yield and easy cultivation are in need.

Cucurbit Genomics Database (Cu-GenDB) is a central portal for genomics, transcriptomics, and genetics of cucurbit species. This breeder-friendly database serves the of storing, mining, analyzing and disseminating these large-scale datasets due to the rapid advances in sequencing technologies, high-quality reference genome sequences of a number of cucurbit crops have been generated and released and to provide a central portal for the cucurbit research and breeding community. Approximately 1.74 million EST sequences have been collected in CuGenDB for four cucurbit species, among which 129,240, 513,801, 588,800, and 508,456 are from melon, cucumber, watermelon, and Cucurbita pepo, respectively, and A total of 21 published genetic maps have been collected for cucurbit species, including 15 for melon, four for cucumber, and two for watermelon (Zheng et al. 2019). Conventional genetic breeding has played an essential role in cucumber cultivar innovation over the past decades. However, it has to combine with the current status of molecular breeding, encompassing the progress and achievements on cucurbits genomics, molecular mechanism underlying important agronomic traits, and also on the creation of high-quality multi-resistant germplasm resources, new variety breeding and ecological breeding.

The preconditions for a successful MAS and MABC are the availability of well-saturated genetic linkage map, tight linkage between the marker and the gene of interest, adequate recombination between the marker and rest of the genome, and the ability to screen a large number of individuals in less time and cost-effective manner. Although numerous QTL mapping studies have identified genomic regions influencing a variety of traits of interest in cucurbits breeding for abiotic tolerance traits, few marker-assisted breeding programs have made use of these findings. For example, in cucumber, the detection of QTLs related to abiotic tolerance traits is very limited. QTL analysis for low temperature germination (LTG) ability is an ideal trait that would allow for early planting. Results from this study that has been conducted using a recombinant inbred line (RIL) population derived from the cross between the LTG tolerant variety and the sensitive, provide a basis for further fine mapping and functional study of QTL for low temperature germination in cucumber. The knowledge is also useful for marker-assisted selection in breeding for this trait (Song et al. 2018; Yagcioglu et al. 2019). With the understanding of nature of inheritance and availability of closely linked molecular markers, it is now possible to develop cucumber genotypes with LTG resistance very rapidly through marker-assisted backcross (MABC) breeding programme.

Germplasm characteristics and DUS

Plant breeding's aim is the ability to withstand abiotic and biotic stresses and superior product quality all depend on genetic variation for key agronomic and horticultural traits, as a result the improvement in crop yield. In search of such variation, breeders frequently shift to germplasm collections to discover new sources of valuable characteristics, especially tolerances to diseases, insects, and environmental stresses such as heat, drought, salt, or cold. In cucumber, there is a detailed genetic analysis of the germplasm collection maintained by the US NPGS, which includes more than 1200 accessions collected throughout the world. The information provided by the GBS data has provided deep insight into the diversity present within the collection and genetic relationships among the accessions (Wang et al. 2018).

To recognize breeder's intellectual property rights, new varieties must satisfy three criteria in the plant variety protection (PVP) system: distinctness, uniformity and stability (DUS). The current DUS testing needs a phenotypic evaluation, which means laborious and time-consuming efforts. Consequently, molecular markers has been considered as an alternative to improve the efficiency and accuracy of DUS testing. This is the first large collection of SNPs was generated for three major pumpkin species (*C. maxima*, *C. moschata*, and *C. pepo*) using GBS and commercial F1 cultivars. These SNPs contribute to an expansion of genomic resources for both basic and applied researches in cultivated pumpkin, providing a rapid and accurate option for variety identification and facilitate development of a DNA-based system for DUS testing in the PVP system (Nguyen et al. 2020).

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Gene pyramiding

The interest of the breeders is the transfer or introgression of many desirable genes from different sources into a variety for genetic improvement. The procedure of mixing desirable traits is known as gene pyramiding and the core of the concept is the development of new crop varieties with few to several different oligo genes for durable disease resistance. Gene pyramiding is a process in which many desirable genes from different parents are assembled into a single genotype. The introgression of multiple QTLs/genes and its effects have been proposed in crop species like wheat, barley, rice, soybean (Rana et al. 2018) and tomato (Hanson et al. 2016), in cucurbits, there are many reports of gene pyramiding, that they use molecular markers to pyramid genes for resistance to biotic tolerance. Pyramiding gene for enhancing tolerance in abiotic stresses is not so extended yet in cucurbits, but there are QTL analyses of abiotic tolerance traits, which can allow for the development of markers for marker assisted selection, focusing phenotypic selection on population subsets containing desired markers and facilitating gene pyramiding for complex traits, such as this report (Yagcioglu et al. 2019). Pyramiding genes to obtain higher and longer-lasting resistance, most of which have to be introgressed from other species, is an important ongoing challenge facing breeders of *C. pepo* (Paris n.d.).

Limitations and prospects of MAS and MABCB

Molecular markers are beneficial in a variety of ways to help plant breeding programs like detection of allelic variations that exist for a gene and are responsible for expression of the traits due to the presence of genetic linkage, marker-assisted procedures for germplasm improvement and varietal development, incorporating multiple genes (gene pyramiding) for resistance to biotic and abiotic stresses into the elite variety, germplasm characterization, genetic diagnostics, characterization of transformants, and the study of genome organization as well as phylogenetic relationships (Cutright 2001).

Compared with conventional breeding approaches, marker-assisted breeding requires more complicated equipment and facilities and the pre-requisites for an efficient marker-assisted breeding program could be considered as limitations. These kind of limitations are referred below:

- The appropriate marker System and reliable Markers. The suitable markers should have the following characteristics: (a) ease and low-cost of use and analysis (b) small amount of DNA required (c) co-dominance (d) repeatability/reproducibility of results (e) high levels of polymorphism, and (f) occurrence and even distribution genomewide (Jiang 2013a, b).
- Not all markers are breeder-friendly. This problem may be solved by converting non-breeder-friendly markers to other types of breeder-friendly markers (e.g. RFLP to STS, sequence tagged site, and RAPD to SCAR, sequence characterized amplified region) (Jiang 2013a, b).
- All markers can not be applicable across populations due to lack of marker polymorphism or reliable marker-trait association. Multiple mapping populations are helpful for a better understanding of marker allelic diversity and genetic background effects (Jiang 2013a, b). In addition, QTL positions and effects also need to be validated and re-estimated by breeders in their own germplasm of interest (Heffner et al. 2009).
- Recombination between the markers and the genes/QTLs of interest can lead to false selection. Use of flanking markers or more markers for the target gene/QTL can help to perform reliable selection (Jiang 2013a, b).
- Inaccurate estimations of QTL locations and effects result in slower progress than expected. The efficiency of QTL detection is attributed to multiple factors, such as algorithms, mapping methods, number of polymorphic markers, and population type and size. Fine mapping with high marker density and in large populations and well-designed phenotyping across multiple environments may provide more accurate estimates of QTL locations and effects (Jiang 2013a, b).
- The most major factor that limits the implementation of MAS is the cost of utilizing markers and the lack of a well-equipped lab which lets to accomplish a large-scale adoption of MAB in practice.

The cucurbits family is characterized by high genetic diversity for fruit shape and other fruit characteristics, resulting in a variety of uses. Thus, as much more understanding and researching the molecular processes in cucurbits and advancement of biochemical and molecular techniques, plant breeding can remarkably contribute towards crop improvement. Currently, high-throughput genotyping techniques allow the breeder to utilize marker-assisted genes screening of many genotypes and shift from conventional breeding to marker-aided selection (MAS). Surely, the major prospect of MAS is its wide application in order to create new varieties response to human and environmental demands. However, improvements in statistical mapping software (new high-density maps) and innovative strategies to boost MAS into breeding programs may lead to a larger role for MAS in plant breeding. As it is written in this paper “Quantitative variation is evident at each of these stages, and is supplying us with the knowledge that, when combined with proper statistical attention, is unprecedented in its power to unravel the inner workings of a genome” (Doerge 2002). For example, additional fine genetic mapping is needed to confirm this discrepancies and similarities for the three QTL detected in the two studies (Song et al. 2018; Yagcioglu et al. 2019).

6.6 Cucurbit Genomics

One major breakthrough in cucurbit research has been the recent availability of the genome sequences of the three leading crop genera in the family, *Cucumis* including cucumber (Huang et al. 2009) and melon (Garcia-Mas et al. 2012), *Citrullus* watermelon (2013), *Cucurbita* squash (Montero-Pau et al. 2018). The advent of next-generation sequencing (NGS) technologies allowed an early completion of the sequence of three cucurbit genomes, cucumber being the first plant genome that was published using mainly NGS. The relatively small size of the genomes of cucumber, melon, watermelon and squash made it possible to obtain draft genome sequences of enough quality to be used for addressing several fundamental biological questions and for their application in plant breeding.

The study of the genetic diversity in natural populations has also been addressed from a genomic perspective in cucumber, melon and watermelon, where 115, 8 and 20 representative accessions have been re-sequenced, respectively. This data offers a large source of variation with enormous potential for mining new alleles and for uncovering genes underlying traits that were domesticated and selected in these species. It is also expected that the number of re-sequenced accessions will grow exponentially during the next years, an issue that is related with the above-mentioned need for cucurbit germplasm characterization.

The first completed genome project of the Cucurbitaceae belongs to the cucumber plant. Seven chromosomes of cucumber have been sequenced using a combination of two techniques including conventional Sanger sequencing and next-generation Illumina sequencing in cucumber cultivar *C. sativus* var. *sativus* L., known as Chinese long inbred line 9930 (Huang et al. 2009). Although high genome coverage (about 72.2-fold) has been obtained, only a small quantity of genes has been identified because of there was limited information about whole genome and tandem duplications at that time. Approximately, 26,682 genes were predicted in the assembled genome of cucumber, which was 243.5 Mb in length. According to flow cytometry analysis of isolated nuclei, the actual cucumber genome size has been calculated as 367 Mb in length (Arumuganathan and Earle 1991). Therefore the assembled genome of cucumber is almost 30% smaller than its actual genome size. For gene prediction, different methods were used including cDNA-EST, homology based and ab initio. About 82% of the genes have been functionally classified or their homologs have been found in related databases such as TrEMBL and InterPro. Furthermore, RNA molecules such as ribosomal RNA, transfer RNA, small nucleolar RNA, small nuclear RNA, and microRNA (miRNA) genes have been identified. About 15,669 gene families have been predicted. A total of 4362 and 3784 families belong to cucumber unique families and single-gene families, respectively. The highest rate of synteny was observed between cucumber and papaya with 9842 syntenic blocks.

Qi et al. 2013 reported a map of genome variation for cucumber that encompasses ~3.6 million variants, generated by deep resequencing of 115 cucumber lines sampled from 3342 accessions worldwide. Furthermore, they found 3,305,010 SNPs, 336,081 small insertions and deletions (indels; shorter than 5 bp) and 594 presence-absence variations (PAVs) when aligned with the reference genome of the inbred cucumber line 9930. 74,166 nonsynonymous SNPs in 19,087 genes, including 1713 nonsense SNPs in 1516 genes causing start codon changes were detected. They concluded that the 115 cucumber lines can be classified into 4 geographic groups.

Recently, 56 divergent artificially selected genotypes of cucumber inbred lines were analyzed using genome resequencing, in order to find sequence variations related to traits of interest (Liu et al. 2019). They identified ~8.4 million single nucleotide polymorphism (SNPs) in comparison to the reference genome of the inbred cucumber line 9930.

The second genome of cucurbits sequenced is the genome of melon (Garcia-Mas et al. 2012). The homozygous DHL92 double-haploid line melon cultivar was sequenced with 454 pyrosequencing and the assembled genome size was about 375 Mb, which represents 83.3% of the melon genome. Moreover, 27,427 protein coding regions have been identified. In order to identify proteins, orthology groups, and metabolic pathways they used an automatic pipe-line for gene annotation. They reported the prediction of 411R-genes which are associated to disease resistance. Some of them contained the nucleotide-binding site and leucine-rich repeat (NBS-LRR) and Toll interleukin receptor domains, which are related to canonical disease resistance for cytoplasmic proteins. In addition, they found genes associated with fruit quality, taste, flavor and aroma. All these genes play pivotal role in sugar and carotenoid concentration, exerting influence on taste and flesh color of melons, respectively. Syntenic relationships between melon and cucumber were studied (Huang et al. 2009; Li et al. 2011). A great level of synteny at higher resolution between melon and cucumber genomes was reported leading to easier detection of small regions in chromosomes. Five melon chromosomes aligned with cucumber chromosomes with several inter- and intra-chromosome rearrangements. Sequencing of other cucurbits members as well as identification and purification of the physical maps are of paramount importance in order to gain a better understanding of genome evolution of the Cucurbitaceae family.

In order to obtain more information about crop evolution, population genomic data of diverse genotypes consisting of elite material, landraces and wild progenitors are required (Schreiber et al. 2018). Resequencing data of 134 wild and 1041 cultivated melon genotypes, were used to construct a SNPs map, which covered the most of the diversity (Zhao et al. (2019). Phylogenetic analyses classified melon accessions into three clades (African, *melo* and *agrestis*). Zhao et al. (2019) using traceability studies for traits of selection combined with QTL mapping in two experimental populations reported genes related to domestication and crop breeding such as genes associated with fruit quality traits. In the context of domestication, loss of bitterness is the most crucial proof of the effect of traits selection in Cucurbitaceae species (Chomicki et al. 2019).

Watermelon genome size is about 425 Mb as reported by Arumuganathan and Earle (1991) using flow cytometry analysis. Guo et al. (2013), sequenced the genome of Chinese elite watermelon cultivar 97103 ($2n = 2x = 22$) using Illumina technology. Coverage was 108.6-fold with 353.5 Mb sequenced, representing 83.2% of the watermelon genome. Because the same pattern of unassembled reads with transposable elements was shown, 16.8% of the watermelon genome was not covered. 23,440 protein-coding genes were identified in the watermelon genome and match to genes of cucumber and melon. Furthermore, R-genes, including NBS-LRR, RLK, and lipoxygenase (LOX), as well as genes related to fruit development, quality, and sugar content were detected. The analyses went one step further, using RNA-seq technique in order to apply expression analysis of these genes at different stages of fruit development using. 20 watermelon accessions (10 from *C. lanatus* subsp. *vulgaris*, six from semi-wild *C. lanatus* subsp. *mucosospermus*, and four from wild *C. lanatus* subsp. *lanatus*) were also sequenced in the context of watermelon genome project. Genetic diversity and population structure of *C. lanatus* germplasms as well as SNPs and indels (insertions/deletions) regions were estimated. Syntenic relationships between watermelon, cucumber, melon, and grape were analyzed, so as to achieve better understanding of cucurbits genome structure. The watermelon and grape genome were about 60% orthologous, which is justified by their genetic similarity. A detailed analysis of each chromosome of watermelon, cucumber, and melon was also conducted. Chromosome analysis revealed high orthologous relationships between Cucurbitaceae family members. Watermelon cultivar '97103' was sequenced using PacBio long reads and analyzed with BioNano optical and Hi-C chromatin interaction maps (Guo et al. 2018). It was the first attempt of a more detailed assembly of genome sequences of watermelon. Subsequently, the same team conducted resequencing and population genetics analysis in 414 watermelon, including 15 *C. colocynthis*, 31 *C. amarus*, 19 *C. mucosospermus*, 345 *C. lanatus* (258 cultivars and 87 landraces), two *C. rehmii*, one *C. ecirrhosus* and one *C. naudinianus* accessions. Information about the evolutionary history of *Citrullus*, was revealed, proving a putative independent evolution in *Citrullus amarus* and the lineage with *Citrullus lanatus* and *Citrullus mucosospermus*. In sum, 19,725,853 SNPs were revealed, of which 1,100,803 were found in coding regions, causing 502,028 nonsynonymous mutations, 589,735 synonymous mutations, 1031 start codon changes and 6808 stop codon changes. Moreover, the nucleotide diversities (π) in four *Citrullus* species with multiple accessions were evaluated. They estimated that *C. colocynthis* ($\pi = 6.75 \times 10^{-3}$) and *C. amarus* ($\pi = 2.28 \times 10^{-3}$) had greater nucleotide diversity than *C. mucosospermus* ($\pi = 0.792 \times 10^{-3}$) and *C. lanatus* landraces ($\pi = 0.56 \times 10^{-3}$) and cultivars ($\pi = 0.548 \times 10^{-3}$).

Cucurbita pepo is the last species of Cucurbitaceae family to be sequenced (Montero-Pau et al. 2019), although the *Cucurbita* genus (squashes, pumpkins and gourds) comprises very important domesticated species such as *C. pepo*, *C. maxima* and *C. moschata.* A high-quality assembly of the zucchini (*C. pepo*) genome with a size of 263 Mb was conducted. It covered 93.0% of the genome, containing 92% of the conserved BUSCO core gene set. The genome is classified in 20 pseudomolecules that cover 81.4% of the assembly and the analysis was enriched with the construction of a genetic map of 7718 SNPs. The topology of the gene family phylogenies, the karyotype organization and the distribution of 4DTv distances imply that *C. pepo* genome is the result of a whole-genome duplication. Furthermore, a simultaneous analysis of 40 transcriptomes of 12 species of *Cucurbit* genus in comparison with all the other published genomes of the Cucurbitaceae family was conducted. In all

Cucurbita species the whole genome duplication was observed, including *C. maxima* and *C. moschata*, in contrast with the genetically distant cucurbits belonging to the *Cucumis* and *Citrullus* genera.

Transcriptome sequencing of *C. pepo* using NGS detected over 9000 SNPs (Blanca et al. 2011). Recently, *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo* genomes were sequenced and assembled (Montero-Pau et al. 2018; Sun et al. 2017). Seven morphotypes of *C. pepo* with diverse edible fruits, from which four of them belong to subspecies *pepo* (Pumpkin, Vegetable Marrow, Cocozelle, and Zucchini) and three of them belong to subspecies *ovifera* (Acorn, Crookneck, and Scallop) were also resequenced with 33.5x coverage (Xanthopoulou et al. 2019). The total number of SNPs detected in each accession ranged from 636,918 to 2,656,513 in green Zucchini and Crookneck, respectively. The three distant genetically accessions from subsp. *ovifera* (Acorn, Scallop, and Crookneck) produced almost twice as many SNPs in contrast to Zucchini reference genome. This thorough analysis of the genome facilitated SNP detection in cultivated pumpkin. Nguyen et al. (2020) located SNPs in different regions of the genome in three pumpkin species *Cucurbita maxima*, *C. moschata*, and *C. pepo*. 37,869 SNPs were detected using genotyping by sequencing. Additionally, they continued genotyping 188 accessions with 288 SNPs (94 F_1 cultivars, 50 breeding lines, and 44 landraces) using a SNP array-based platform.

The genomics of Cucurbitaceae domestication

To date, 11 reference genomes of cucurbits have been published. Specifically, C. sativus var. sativus cv 9930 and cv Gy14, one wild cucumber (*C. sativus* var. *hardwickii* PI 183967), one cultivated melon (*C. melo* cv DHL92), two cultivated watermelons (*C. lanatus* subsp. *vulgaris* cv 97103 and cv Charleston Gray), four cultivated Cucurbita species (*C. maxima* cv Rimu, *C. moschata* cv Rifu, *C. pepo* cv MU-CU-16, and *C. argyrosperma*), and one cultivated bottle gourd (*Lagenaria siceraria* cv USVL1VR-Ls) (Chomicki et al. 2020 and references therein), with the number of relevant publications increasing exponentially. According to studies between domesticated and wild species in cucurbits, the priority of domestication seems to be the loss of fruit bitterness caused by the presence of toxic cucurbitacin (Zhou et al. 2016). Selection was also focused on the presence of seed coat, carotenoid and sugar content.

We restrict our review of comparative and functional genomics of cucurbit crop traits to traits directly relevant to domestication. Molecular mechanisms underlying flower sex-determination, fruit development, and spine development in cucumber are reviewed by Che and Zhang (2019).

At this point we summarize, the key features whose changes are directly related to domestication.

1. Loss of bitterness

Cucurbits include sweet fruit pulp species, minor crops such as *Cucumis anguria*, *Cucumis metuliferus and Melothria scabra* and bush species found in the wild such as the fruit pulp of wild *Kedrostis foetidissima*, *Momordica* species, *Solena heterophylla*, and some *Trichosanthes* (Chomicki et al. 2020). Fruits of most cucurbit species have a bitter taste caused by a group of terpenoid compounds known as cucurbitacins. Cucurbitacins can be found in abundance in leaves, roots and fruits of wild cucurbits and one of their most important roles is the defense against herbivores. Most species belonging to Cucurbitaceae family have lost their bitterness during domestication, in contrast to some varieties of *Momordica charantia* that still retain their bitterness.

Huang et al. (2009) found that the Bi gene is responsible for bitterness in all parts of the plant. Later, Shang et al. (2014), by performing biochemical and genetic analysis on cucumber, they demonstrated that Bi gene, encoding a cucurbitadienol synthase, catalyzes the first bound step in the biosynthesis of cucurbitacin C. Cucurbitacin biosynthesis is regulated by two bHLH transcription factors which in turn increase the expression of the Bi gene in the leaves (Bl) and fruits (Bt) via binding in the E-box elements of the Bi promoter (Shang et al. 2014). No attempts have been made to silence the Bi gene in cucumber (Qi et al. 2013) because the loss of its biosynthesis in all parts of the plant, even in cultivated varieties, is detrimental due to its involvement in the defense against herbivores. According to studies performed on various cucumber lines with different levels of bitterness, the lack of bitterness during domestication has been shown to result from mutations in the Bt gene in its cis-acting elements or influence of the binding site that caused the downregulation of Bt (Qi et al. 2013; Shang et al. 2014; Zhou et al. 2016). Studies at genomic level of three species that produce cucurbitacin, cucumber (cucurbitacin C), melon (cucurbitacin B) and watermelon (cucurbitacin E) proved that the lack of bitterness is caused by convergent mutations at Bt loci (Shang et al. 2014; Zhou et al. 2016). During domestication, various mutations occur in genes that are responsible for regulating the expression of pathways in specific tissues, helping to avoid pathway related pleiotropic phenomena (Lenser and Theißen 2013).

2. Selection for sweetness

Domestication has contributed to the selection of sweet fruits of various Cucurbitaceae crop species, such as watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*). Guo et al. (2013), studied the genome and transcriptome of watermelon and identified a-galactosidase, insoluble acid invertase, neutral invertase, sucrose phosphate synthase, UDP-glucose 4-epimerase, soluble acid invertase and UDP-galactose/glucose pyrophosphorylase which are the most important enzymes related to sugar content and metabolism during ripening process. They revealed 62 genes related to sugar metabolism of which 13 were differentially expressed in fruit flesh and 76 genes related to sugar transport 14 of which were differentially expressed in fruit flesh. Garcia-Mas et al. (2012), annotated 63 genes controlling sugar metabolism. Guo et al. (2015), studied domesticated watermelon (sweet and red-fleshed) and found that the expression levels of two Sugars Will Eventually be Exported Transporter (SWEET)-like transporters that were too high in the red flesh of the watermelon (as opposed to the rind) are involved in regulating sugar content in fruits by smoothing the way for transmembrane transport of sugars.

Furthermore, Ren et al. (2018), found a tonoplast sugar transporter (*ClTST2*) in watermelon which lays sucrose, glucose and fructose on fruit's vacuoles. Various comparisons between hundreds of species of *Citrullus* accessions proved that the expression of *ClTST2* plays a pivotal role in the domestication of watermelon. A sugar-induced transcription factor called SUSIWM1 is responsible for ClTST2 regulation with a single SNP in the tonoplast sugar transporter (*ClTST2*) alteration causing sweet flavor in domesticated watermelon (Ren et al. 2018). Moreover, they revealed a quantitative trait locus (QTL) in watermelon related to sugar accumulation. Increased *ClTST2* expression by enhanced binding efficiency of SUSIWM1 to the ClTST2 promoter is of paramount importance for the sugar content during the domestication of the watermelon.

Collectively, high sugar content during melon and watermelon domestication is a result of the selection of various genes involved in sugar metabolism and transport.

3. Selection for larger fruits

The choice of humans in the fruits of the 10 most important species of the Cucurbitaceae family was mainly related to the fruit size. The main processes that are responsible for the size of the fruit occurring in the ovary are: cell differentiation, cell division and cell expansion. Cell differentiation refers mainly to carpel number. The number of carpels varies only between 3 and 7 with the oversized species *Cucurbita maxima* consisting of only 5–7 carpels (Grumet and Colle 2016). Thus, changes in fruit size are mainly caused by alterations in cell division and cell expansion. The transcriptional regulators that underlie fruit size and related genes have not revealed yet, but phytohormone-related genes (auxin, cytokinins and gibberellins), microtubule-related genes and cyclin-related genes have found to play a pivotal role in fruit size alteration.

In watermelon, two MADS box genes putative homologues to the tomato ripening and expansion gene *TAGL1* have been revealed and found to have high expression during fruit expansion and ripening, making possible that they play a role in both processes regulation (Guo et al. 2013). Qi et al. (2013), analyzing a QTL in cucumber, revealed 19 genes, one of which encodes a cyclin related to cell proliferation. Furthermore, Weng et al. (2015), found 12 QTLs related to fruit size in cucumber correlated to length and width. Jiang et al. (2015), studied the transcriptome of cucumber and identified various differentially expressed genes, with many of them involved in cell division through microtubules and cyclins. Wang et al. (2017), proved the putative role of spontaneous short fruit 1 (sf1) gene in cucumber length regulation through its involvement in auxin and cytokinin signaling.

Fruit size regulation is a multidisciplinary procedure affecting domestication. *Cucurbita maxima* cv Dill's Atlantic Giant, are the world's largest fruits weighing >1000 kg. Despite the various fruit size and weight germplasm of Cucurbitaceae crops, the regulation of the mechanisms that underlie the differences in fruit size and weight is scarcely understood. A future chapter of great interest is the identification and functional characterization of genes involved in cell division and expansion in fruits (Chomicki et al. 2019).

4. Seed traits

The cultivation of Cucurbitaceae species with the aim of their seeds is rare and is mainly limited to the *Cucurbita pepo* species. The Styrian pumpkin, *C. pepo* subsp. *pepo* var. *styriaca*, is a variety that is cultivated for its seed (Teppner 2004). Another cucurbit cultivated for its seeds is the West African egusi melon, *C. mucosospermus*, because of the nucleotide differences with the domesticated watermelon in genetic loci related to regulation of fatty acid metabolism in seeds (Guo et al. 2013). According to Mendelian genetics a single recessive locus underpins the egusi seed type in watermelon (Gusmini et al. 2004).

5. Selection for high carotenoid content

Many cucurbit crops contain b-carotene or lycopene, which have antioxidant properties that benefit human health. Moreover, many wild species contain high quantities of carotenoids because of the orange or red color. Watermelon and honey melon constitute the main crops studied for their carotenoid concentration which is regulated by various metabolic genes (Garcia-Mas et al. 2012; Guo et al. 2013). Guo et al. (2011, 2015), revealed that phytoene synthase (PSY) and phytoene desaturase (PDS) genes of the carotenoid biosynthetic pathway in domesticated red-flesh watermelons were upregulated.

Grassi et al. (2013), identified 19 transcription factors underpinning lycopene accumulation in watermelon with a minimally expression of lycopene cyclase (the enzyme that converts lycopene into b-carotene). Xishuangbanna cucumber (*Cucumis sativus* var. *xishuangbannanensis*) which is cultivated in China, Laos and Vietnam has also high lycopene concentration as well as high sugar and b-carotene content (Renner 2017). A substitution of alanine to asparagine at position 257 of the BCH1 gene, encodes b-carotene hydroxylase resulting to orange flesh of Xishuangbanna cucumber (Qi et al. 2013). This substitution results to the creation of a nonfunctional b-carotene hydroxylase that cannot convert b-carotene into zeaxanthin, therefore concluding to increased concentration of b-carotene (Qi et al. 2013).

Zhang et al. (2017a, b) revealed that chromoplast-localised phosphate transporter (*ClPHT4;2*) is the key gene for lycopene accumulation. ClPHT4;2 is found in domesticated red-fleshed watermelon and it is highly expressed in contrast to white-fleshed watermelon which is present but it is expressed at a very low level. High expression of ClPHT4;2 is correlated to the presence of cis-acting elements in its promoters that play pivotal role in binding of sugar, abscisic acid and ethylene inducible transcription factors ClbZIP1 and ClbZIP2. White-fleshed watermelon lacks the existence of these elements (Zhang et al. 2017a, b). Zhang et al. (2017a, b), suggested that sweetness and red color traits are interrelated in watermelon.

6. Tendrils

Cucurbitaceae evolution and divergence is influenced by climbing ability, hence Guo et al. (2020) studied the evolution of tendrils. They also revealed that TEN gene plays an important role in the appearance of tendrils. In *Cucurbita* species, two copies of the TEN gene have been identified but their function has not been fully elucidated (Chomicki et al. 2019).

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Chapter 7
Mitigating Abiotic Stresses in *Allium* Under Changing Climatic Scenario

Anil Khar, Hira Singh, and Priyanka Verma

Abstract *Alliums* have an ancient history of domestication and cultivation by human beings. Onion and garlic are the two main members of the genus *Allium* that possess high economic and commercial importance across the globe. Though economically important, yet the research aspects on stresses are largely ignored. Major limitations are huge genome size, biennial life cycle, highly cross-pollinated nature and inbreeding depression in onion. Asexual nature of garlic restricts its genetic improvement through conventional techniques. Hence, genetic improvement of garlic is more unwieldy as compared to onion. Climate change will definitely decrease the production and productivity of *Allium* crops as in other food and feed crops. The center of diversity of the majority of the *Alliums* is Central Asia. Enhanced temperature due to global warming would lead to major abiotic stresses for their cultivation. Development of climate smart varieties against abiotic stresses is the chief and utmost concern for the breeders. Systematic and focused research plan for the development of genetically improved cultivars having wider adaptability and higher yield potential in the swiftly changing climate scenario is needed. Attention towards development of genomic resources, whole genome sequencing and other omics tools along with conventional breeding approaches for Allium genetic improvement are needed. These strategies are mandatory to bridge the gap between demand and supply of Allium crops around the world. In this chapter, we have summarized the research progress made to understand the abiotic stress in Allium and the ways and means to mitigate this global crisis.

Keywords Abiotic stress tolerance · *Allium* · Drought · Genetic resources · Genomics · Molecular breeding · Osmoprotectants · Salinity

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7.1 Introduction

Commercial cultivation of any domesticated crop needs utmost attention towards genetic improvement and agronomic management. The inherited yield potential of any cultivar or variety is not invariably achieved, as various critical aspects and factors like availability and timely application of various agri-inputs, congenial season, climatic conditions, biotic and abiotic stresses specific to the region and crop challenge this potential. With a rapidly changing climate scenario, various abiotic stresses such as water, heat, salt etc. are emerging at a greater pace across the globe. Depending upon the season, geographical location and prevailing environmental conditions, intensity of abiotic stresses influence almost every crop production practice. With the advancement of plant breeding approaches, high yielding cultivars and production technologies have been developed but their adaptability is not widespread under the current situations.

Among abiotic stresses, stresses particularly waterlogging/flood, drought, salinity/alkalinity and thermal stresses (extreme high and low) are the major challenges and constraints for the crop production and agriculture globally. To manage these physiologically, genetically and biochemically complex processes is a real challenge in front of the contemporary plant breeders irrespective of the crop and country, especially for the developing countries. Directly or indirectly these abiotic stresses influence the farmers' livelihoods, ultimately adversely affecting food security and national economy. Globally, one or complex of abiotic stresses directly cause about 70% reduction in crop yield (Acquaah 2007). Furthermore, the global population is rapidly increasing and is likely to reach about 9.8 billion by 2050. On the other hand, food production and crop productivity are at their highest potential and have somewhat plateaued out. There is a big gap between food demand and supply. Augmenting drought and salinization of cultivated land are the indicators of devastating effects on agriculture and crop production (Wang et al. 2003). Globally, drought is a leading abiotic constraint for onion production (Gedam et al. 2021).

Allium, being monocotyledon, is considered the largest genus containing more than 900 species, mainly existing in the Northern Hemisphere. Commercially garlic (*Allium sativum*), onion (*A. cepa*), leek (*A. porrum*), shallot (*A. cepa* L. Aggregatum group), chive (A. *shoenoprasum*), bunching onion (*A. fistulosum*) and others, are the important species, which are extensively consumed by human being since antiquity. *Allium* species are the rich source of biologically active compounds e.g. sulfur derivatives, quercetin, flavonoids, saponins with significant anticancer, cardioprotective, anti-inflammation, antiobesity, antidiabetic, antioxidant, antimicrobial, neuroprotective, and immunomodulating properties (Sugihara et al. 2004; Zeng et al. 2017; Golubkina et al. 2020).

7.1.1 Onion

Onion (*Allium cepa* L., 2*n* = 2*x* = 16) is the major representative of the genus *Allium* and family *Amaryllidaceae* that is commercially grown for fresh and processing purposes across the globe (Hanci and Gökçe 2016; Havey and Ghavami 2018; Khar and Singh 2020). The world production and productivity in the last 20 years has been presented in Fig. 7.1. Being a versatile crop, this is well adapted to a varied range from temperate to tropical climatic conditions. Onion is a shallow rooted and environmental sensitive crop and its establishment, growth and development are negatively affected by extreme climatic situations (Mubarak and Hamdan 2018; Chaudhry et al. 2020). Various abiotic stresses such as temperature, moisture, salt and heavy metals are the major threats of onion production, productivity and other quality and nutritional traits (Ardahanlioglu et al. 2003; Sönmez et al. 2005; Kendirli et al. 2005; Cemek et al. 2007; Bilgili et al. 2011; Hanci and Cebeci 2015; Chaudhry et al. 2020). Like other crops, breeding and genetic studies related to abiotic stress

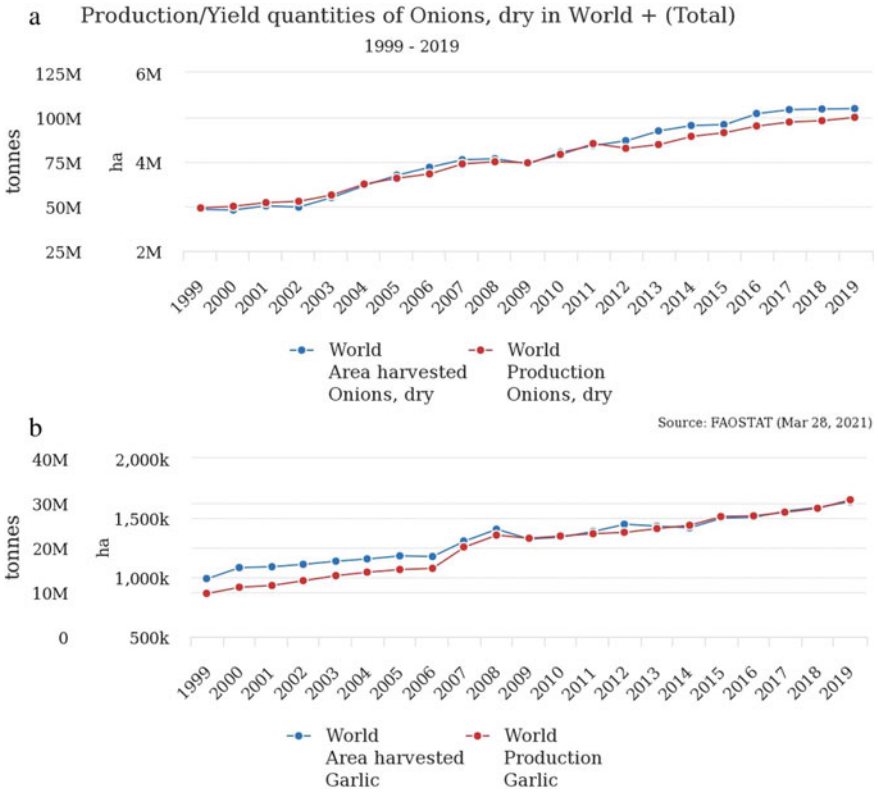

Fig. 7.1 Global area and production (million tons) of onion (**a**) and garlic (**b**) from 1999 to 2019 (Source –FAO 2012)

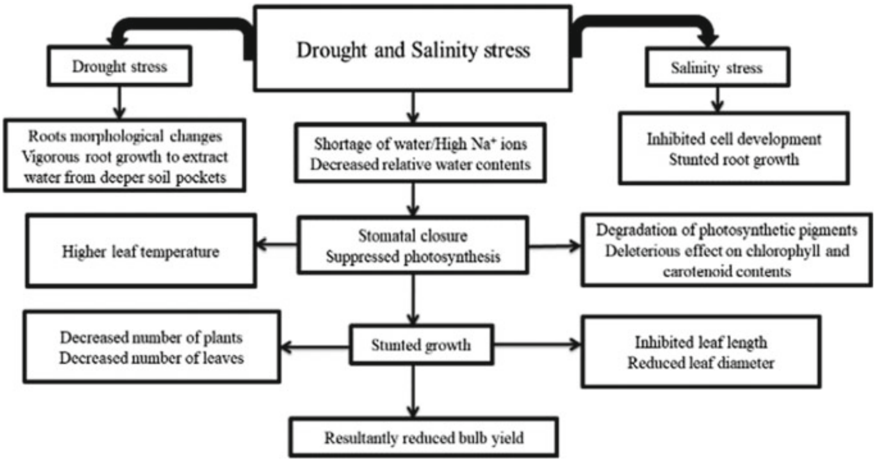

Fig. 7.2 Common effects of salinity and drought stresses on onion crop (Adapted from Chaudhry et al. 2020)

mechanisms and effects are very limited in onion. Huge genome size, availability of less genomic resources, biennial life cycle, seasonal requirement for bulb formation, high inbreeding and outcrossing nature (Ricroch et al. 2005; Shigyo and Kik 2008; Havey 2012; Khar and Singh 2020) are the major constraints for its breeding and genetic studies. Common effects of salinity and drought stresses on onion crop have been presented and summarized in Fig. 7.2 (Adapted from Chaudhry et al. 2020).

7.1.2 Garlic

Garlic (*Allium sativum* L.), being the oldest domesticated horticultural crop, was an indispensable diet component of Egyptian working and labor classes to maintain and increase their physical strength, thereby, enabling them to work harder (Moyers 1996; Mostafa et al. 2013; Abdelrahman et al. 2016, 2019). This bulbous crop had been used as an antiseptic to cure gangrene during the First World War (Hedrick 1972). Presently, about 90% garlic production is from Asia particularly China and India. The world production and productivity of garlic in the last 20 years has been presented in Fig. 7.1. A strictly asexually propagated crop, it possesses low genetic variation and diversity. This is the major hindrance for the development of widely adapted and genetically improved cultivars through conventional breeding approaches. In the modern omics era, exiguous genomic and molecular studies have been carried out in this ancient crop compared to other vegetables. The genome size of garlic is 15.9 GB (Arumuganathan and Earle 1991; Jones et al. 2004; Abdelrahman et al. 2017) that is almost equivalent to wheat genome. To develop new cultivars having potential to combat with various abiotic stresses, breeders have options like meristem culture, mutagenesis, genetic transformation, and molecular breeding. Scanty research has been conducted on these aspects in garlic. Most of the cultivars being developed by the public sector are through clonal selection that are susceptible to several biotic and abiotic stresses (Sako et al. 1991; Conci et al. 1992).

Under changing climatic scenarios, like other crops, garlic production is also vulnerable to various abiotic stresses. However, no systematic studies have been documented yet. Reddy et al. (2000) elicited that crop production is decreasing over the years because of rapid climate change. Yield component of garlic is susceptible to environmental conditions (Panse et al. 2013). Mutations (random or induced), somaclonal variations and genetic transformation are the main source of genetic variations (Novak 1990; Burba et al. 1993; Rubatzky and Yamaguchi 1997; Robinson 2007; Sandhu et al. 2015; Singh et al. 2021a, b). Clonal propagation is the major constraint for inculcating economically important traits especially for the tolerance of abiotic stresses and more yield (Kamenetsky 2007). Even though marker assisted breeding is also challenging owing to the larger genome size and its highly complexity (Kamenetsky et al. 2015; Egea et al. 2017).

7.2 Diversity Studies for Abiotic Stress Breeding

Due to phenotypic plasticity, garlic displays widespread range of morphological variation in terms of bulb size, number of leaves, length of floral scape, clove size, number and arrangement, and inflorescences (Pooler and Simon 1993; Keller 2002; Kamenetsky et al. 2005; Buso et al. 2008). Various reports of garlic diversity study (Pooler and Simon 1993; Etoh et al. 2001; Lampasona et al. 2003; Zhao et al. 2011; Khar et al. 2020; Jo et al. 2012; Hirata et al. 2016) have been documented. Regarding abiotic stresses, very less systematic research work has been carried out. Kamenetsky et al. (2005) stated that garlic collected from the place of origin possesses superior traits including tolerance to various abiotic stresses. Although, having great commercial and health beneficial importance, not much research attention or focus has been put for the germplasm collection, preservation, and conservation since long which has led to the shortage of germplasm (Rabinowitch and Zeltzer 1984; Kamenetsky 1993, 2007; Baitulin et al. 2000; Fritsch 2001; Keller and Senula 2001). The precious local gene pool is currently under severe threat of extinction, due to the rapid replacement of traditional landraces with modern cultivars (Kamenetsky et al. 2005; Ovesna et al. 2011).

7.2.1 Water Stress Tolerance

Water stress i.e., drought or waterlogging is a critical constraint or challenge for the crop growth and development. Being short duration crops, these conditions become more cumbersome for the vegetable crops. With the changing climatic conditions, such problems are increasing in the different areas globally that ultimately affect the crop yield and productivity adversely. Among vegetable crops, garlic crop shows considerable decline in chlorophylls (a, b and total), anthocyanin, carotenoids, vegetative growth parameters such as plant and root fresh weight, yield of bulb, various quality parameters and higher allicin amount and enhanced ion leakage under water deficit conditions (Bideshki et al. 2013; Diriba-Shiferaw 2016). Waterlogging or excess rainfall situation is also devastating for the garlic growth and bulb formation (Diriba-Shiferaw 2016). Csiszár et al. (2007) recorded actions of antioxidant enzyme in three *Allium* spp. under drought conditions and found that after one-week manipulations in the activities of enzymes related to glutathione (GR, GST) and POD in shoots linked with relative water content of leaves. Some inducible antioxidants played a major role under drought conditions in some *Allium* populations. These studies might be beneficial to develop widely adapted climate-smart garlic varieties. Genotypic difference towards the tolerance mechanism was observed by Badran (2015) in Egyptian garlic cultivars. Egaseed 1 was most tolerant while Balady was the highly drought sensitive genotype based on superiority measure, drought tolerance index, percent yield injury and relative performance.

Being a shallow rooted crop, onion is highly sensitive to water stress both in excess and deficit along with temperature (Brouwer et al. 1989; Pelter et al. 2004; Balla et al. 2013; Ghodke et al. 2018a, b; Gedam et al. 2021). About 30% yield losses were recorded under drought stress in onion (Potopova et al. 2016). Drought tolerance is one of the genetically, morphologically, biochemically and physiologically complex phenomena controlled by several genes. Recently, Gedam et al. (2021) screened 100 onion genotypes for drought tolerance through multivariate analysis. All genotypes were assessed on the basis of the drought tolerance efficiency (DTE), percent decline in bulb yield, and multivariate analysis results. Yield of onion bulbs was positively correlated with relative water content (RWC), membrane stability index (MSI), total chlorophyll content, leaf area and antioxidant enzyme activity under drought stress conditions. Tolerant genotypes exhibited higher DTE (>90%), lesser loss of yield (<20%), while the highly sensitive genotypes displayed > 70% yield loss. Differential physiological and biochemical response in drought sensitive and tolerant genotypes in onion have been presented in Fig. 7.3.

7.2.2 Salinity Tolerance

Worldwide, salt stress is continuously threatening the crop production and productivity at a greater pace. Very few focused research attempts have been conducted in the garlic related to salt stress. For the development of climate smart garlic cultivars especially under salinity conditions, information regarding the tolerance of salt stress levels is very important to screen the suitable genotypes. Silenzi et al. (1985) recommended that salt levels from 0.96 to 5.40 $\mathrm{dSm^{-1}}$ affect sprouting of cloves. However, no effect was noticed on the final sprouting of cloves. Mangal et al. (1990) found reduction of half yield because of salt stress from 5.60 to 7.80 $\mathrm{dSm^{-1}}$, specific to the

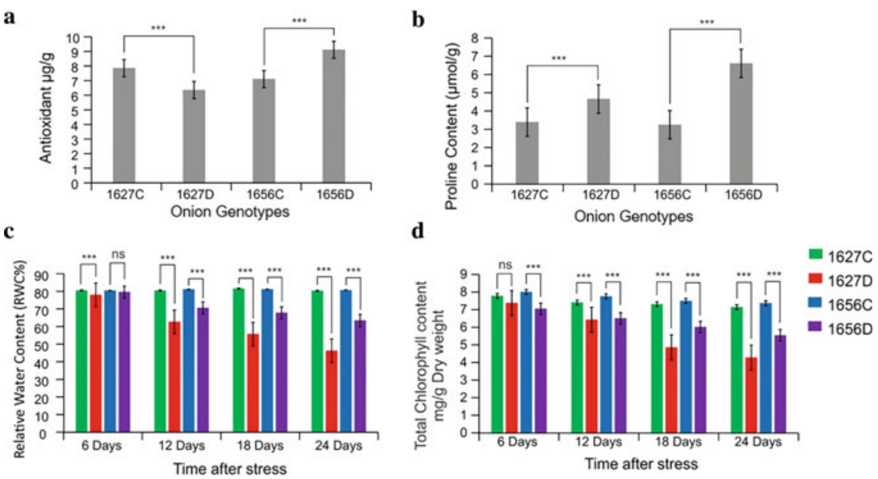

Fig. 7.3 Differential physiological and biochemical response in drought sensitive (1627) and tolerant (1656) onion genotypes. **a** Total antioxidant, **b** proline content, **c** relative water content and **d** total chlorophyll content (Adapted from Ghodke et al. 2020)

garlic genotype. Likewise, they further indicated that if soil salt content surpasses 1.70 dSm^{-1}, the yield reduction was observed by 1.68% per unit increase in soil salinity. Francois (1994) signaled a tolerance threshold of 3.9 dSm^{-1} and a 14.3% yield reduction for each unit increase in salinity above the threshold. Although salt tolerance threshold was slightly higher in garlic than other vegetable crops, yields reduction is rapid once the soil salinity values exceed the threshold (Maas and Hoffman 1977).

In the case of onion, reduction in bulb sprouting, inhibition of vegetative growth and decline in onion seed yield was recorded with enhanced osmotic pressure in the root zone because of effects of high concentration of salts (Mangal et al. 1989). In Turkey, Chaudhry et al. (2020) observed that both stresses significantly reduce photosynthetic rate, leaf length, leaf number and bulb yield. Root parameters were affected differently under both stresses; salinity affected root diameter and length, while drought caused elongated and thicker roots. They further observed that the stress effect is genotypic specific which is very important to know sensibility level. They categorized the cultivars into tolerant and susceptible on the basis of overall performance under both stresses. Such screening studies could be quite useful for further breeding programs and for further omics studies. Semida et al. (2016) sprayed 0.5 mM α-tocopherol on two onion cultivars to mitigate the effects of soil salinity stress (8.81 dSm^{-1}). Foliar application of α-tocopherol significantly enhanced salt stress tolerance by reducing the endogenous H_2O_2 and lipid peroxidation, and enhancing enzymatic (glutathione reductase, catalase, superoxide dismutase, ascorbate peroxidase) and non-enzymatic (glutathione and ascorbic acid) antioxidant activities. Further, they reported that there was significant influence on photosynthesis efficiency and plant water status in terms of membrane stability index and relative water content. Hence, application of α-tocopherol as foliar application could be an alternate option to induce salt-tolerance in onion.

7.2.3 Thermal Stress and Photoperiod

Among various abiotic stresses, thermal stress is very critical for all crop plants that adversely influences germination, growth and development of plant, metabolism and biochemical pathways, and ultimately yield. Both high and low temperature affects various biochemical pathways and enzymatic functions. Degree and deviation of optimum required temperature are critical for the plant response severity. For sustaining crop production, thermal stress is becoming a major issue for policy makers and plant breeders under rapid changing climate scenarios.

Likewise, all crops, alliums are also highly influenced by temperature and photoperiod. These environmental parameters are very critical for the overall plant growth of all alliums (Takagi 1990; Pooler and Simon 1993; Brewster 1994; Etoh and Simon 2002; Kamenetsky et al. 2004). In garlic, lower temperature encourages development of floral parts and extended photoperiod is crucial for floral scape elongation (Takagi 1990). Changeover of the vegetative to a reproductive state occurs during the active growing phase (Kamenetsky and Rabinowitch 2001). Higher temperature along with extended photoperiod enhanced the reserve translocation to the cloves, and the degeneration of the developing inflorescence (Kamenetsky et al. 2004). In bolting garlic genotypes, deviation of the environmental parameters like temperature, before and after planting, could regulate the flower development and restoration of fertility. Wu et al. (2016) exhibited that presence of more amount of endogenous plant hormones like GA and MeJA have positive impact for bulb formation and bolting that changed with several treatment combinations of temperature and photoperiod. At a molecular level, Son et al. (2012) identified four downregulated and fifteen upregulated genes that were responsive to low temperature or cold responsive (CR) genes. Such CR genes could be modified to alleviate cold or frost stress during its hibernation in field conditions.

Onion production especially in tropics is highly dependent on temperature and moisture. Low-temperature is required during the early development of onion bulbs. Higher temperature adversely affects bulb formation and overall plant development of the plant and also leads to bulb splitting (Abdalla 1967; Khokhar 2017). Higher soil temperature causes elongation of bulbs which is undesirable economically (Yamaguchi et al. 1975). With changing climate, high temperature would be the biggest challenge for onion production especially in tropical conditions (Pathak 1994). Night temperatures below 10 °C for a 2–3-week period induces bolting (seed stalk formation) in onions after the 7–10 leaf stage. However, little bolting occurs if temperatures are around 21 °C. High temperatures during early growth also induces bolting in onion. The optimum temperatures for ideal onion growth and development, before bulbing and bulb development are 20–25 °C, 13–24 °C, 15–21 °C and 20–25 °C, respectively (Rao 2016). Certain temperature fluctuations lead to early maturity eventually reducing bulb size and economic yield (Butt 1968; Brewster 1979; Seabrook 2005).

7.2.4 Heavy Metals

Soil contamination with various toxic heavy metals has been an important environmental issue owing to its utmost adverse impacts on overall ecosystems (Yang et al. 2015). Among all heavy metals, lead (Pb) is the biggest problem for the environment due to various anthropogenic activities with the onset of modern agriculture and industry (Lyu et al. 2020). Excess use of Pb leads to toxicity in the living system of animals and plants (Liu et al. 2009). Pb toxicity causes decline in seed germination (Wierzbicka and Obidzinska 1998), abnormalities in mitosis process (Levan 1945; Wierzbicka 1994), toxicity of nucleoli (Liu et al. 1994), leaf chlorosis (Johnson and Proctor 1977), inhibition of plant growth (Fargasova 1994), photosynthesis reduction (Poskuta and WaclawczykLach 1995), replication process (Gabara et al. 1992), enzyme activities and functions (Hampp et al. 1973), metabolic disorders and even death of plant (Yang et al. 2015) and eventually reduction of overall plant growth and crop yield (Seaward and Richardson 1990).

Such heavy metals produce free radicals which cause oxidative stress in plant systems (Dietz et al. 1999). In garlic, Pb in excess adversely affects various physiological activities and processes. Liu et al. (2009) investigated the effect of Pb in garlic and they revealed the vital role of inducible antioxidant enzymes in garlic under lead stress condition and exhibited a direct relationship between lead accumulation in plants and inducible antioxidant enzyme activities. Their results pointed out that both shoot and root growth inhibited considerably at high concentration of Pb and maximum lead accumulation determined in roots compared to shoots and bulbs.

Recently in China, Lyu et al. (2020) studied the molecular mechanism of lead stress response in onion in terms of mitosis, replication, gene expression and proteins. Their observations revealed that roots were highly sensitive to lead stress. At DNA level, aberrations in chromosomes as well as reduction in mitotic index with different concentrations of lead were noticed. More DNA damage and suppressed cell cycle progression had been observed with increasing lead concentration and duration of exposure. A total of 17 proteins were expressed differentially between lead treated groups and control. Declined expression of Anx D1 exhibited reduction in defensive response whereas SHMT1 reducing expression led to lower respiration rate. Decreased expression of COMT2 resulted in decreased response of other functions and the enhanced expression of NDPK suggested higher transcription and protein synthesis. Increased expression of PR1 and CHI1 controlled more pathogen invasion whereas augmented expression of MPK5 and ORC5 lead to the declined replication process. Decreased expression of POLD1 indicated reduced DNA repair activity. These results could be milestones and new insights at the proteomic level for alliums to study lead stress and development of new molecular markers to detect Pb toxicity response at early stage.

7.3 Use of Arbuscular Mycorrhizal Fungi (AMF) for Abiotic Stresses

Arbuscular mycorrhizal fungi (AMF) are the important soil possessing beneficial microorganisms. They play a great role, for most of the terrestrial plants, by developing symbiotic relationships which have positive impact for enhancing crop yield and quality under adverse environmental growing conditions by reduction in the use of mineral fertilizers and pesticides (Caruso et al. 2018; Golubkina et al. 2019). Use of AMF could be a great alternate option for enhancing tolerance for heavy metal by increasing plant nutrient acquisition (Sheng et al. 2008; Wang et al. 2012). Alliums respond well towards AMF owing to a shallow rooted system (Bowling et al. 1980; Greenwood et al. 1982; Sanders and Fitter 1992; Bever et al. 1996; Deressa and Schenk 2008; Golubkina et al. 2020).

7.4 Application of Osmoprotectants for Abiotic Stresses

Exogenous application of different osmoprotectants like proline could be a great alternate option for inculcating tolerance for drought, salinity etc. in plant species by enhancing WUE, and improvement of antioxidant defense system (Rady et al. 2015; Nahar et al. 2016; Semida et al. 2017; Noreen et al. 2018; Gholami and Ehsanzadeh 2018; Hossain et al. 2019). Alliums possessing a shallow-root system and use only 20 cm of soil depth for its growth and development (Brewster 2008). Therefore, allium species are very responsive to water stress (both excess or deficit), need proper and regular irrigation for sustainable production of bulbs especially in tropical and subtropical conditions (Zheng et al. 2013; Rao 2016; Semida et al. 2017; Abdelkhalik et al. 2019a, b). In arid/semiarid regions, salinity, high temperature and calcareous soils are cumbersome and challenging for the allium production due to more evapotranspiration and insufficient rainfall (Tripathi and Müller 2015). During this stress, a specific plant defense mechanism against the stresses is the generation of complex antioxidant defense system and further accumulation of osmoprotectants such as amino acids (proline), betaines (glycine betaine), polyamines and sugars (trehalose, polymers, sorbitol) (Hossain et al. 2019; Rady et al. 2018; Semida et al. 2019). During drought stress, application of proline as osmoprotectant accumulates in cells, that functions as an osmolyte for osmotic adjustment, stabilizes sub-cellular structure, reduces oxidation of lipid membranes or photo-inhibition, scavenging ROS and cellular redox buffering capacity (Ashraf and Foolad 2007; Hayat et al. 2012; Heuer 2010). Thereby, resulting in mitigating the adverse effects of stress (Hossain et al. 2019; Semida et al. 2020).

Recently in Egypt, Semida et al. (2020) evaluated and assessed the effects of exogenous application of proline (1 and 2 mM) under water stress conditions on seedling growth, physio-biochemical traits, plant defense system, bulb yield and

WUE of onion grown on calcareous and salty soil. They observed that proline application significantly increased growth, photosynthesis, plant water status and osmoprotectants of drought-stressed onion which accordingly give higher bulb yield. Further, they noticed that foliar application of proline might decrease moisture deficit stress by augmenting sugar content as well as strengthening the self-defense system of plants.

7.5 Soil Salinity and Salt Stress

Flooding, waterlogging, and salinity harshly influence the yield of various Allium crops (Yiu et al. 2009; Sta-Baba et al. 2010) and onions are categorized as the highly salt sensitive crop category (Shannon and Grieve 1999). Presently, across the globe, a loss of about 3 million ha of arable and cultivated land every minute owing to salt and soil salinity is reported. Rapid change of climatic conditions, boost the severity and frequency of abiotic stresses, specifically salinity, drought, and thermal stress with significant loss of yield in various plant species (Lobell and Field 2007).

Endogenous biosynthesis of glycine betaine (GB) is documented during the salt stress period of plants, but its production amount and accumulation depends only on the degree of salt tolerance of the particular crop plants (Sakamoto and Murata 2000). Thus, application of exogenous GB on plants could be used to enhance salt tolerance (Hayashi et al. 1998; Cha-Um and Kirdmanee 2010; Kaya et al. 2013; Alasvandyari et al. 2017), since these GB sustain osmotic regulation in the salt stressed plant cells (Gadallah 1999). GBs also help various transporters for functioning normally as well as maintaining higher activities of antioxidant enzymes and reduction of adverse effect of oxidative stresses (Gadallah 1999; Rahman et al. 2002; Ma et al. 2006; Hu et al. 2012; Alasvandyari et al. 2017; Alasvandyari et al. 2017).

In onion, Rady et al. (2018) investigated the protective role of foliar application of three levels (0, 25 and 50 mM) of glycine betaine (GB) against 4.80 dSm^{-1} salt stress in the form of growth and yield performances, WUE, tissue health, osmoprotectant contents, antioxidant defense system activities. Treatment of GB significantly enhanced shoot length, leaf area, fresh and dry weight of shoot, bulb yield, WUE, chlorophyll amount and their efficiency, stomatal conductance and tissue health measured as relative water content and membrane stability index.

7.6 Interspecific Hybridization

The genus *Allium* is considered to be the oldest domesticated species comprising onion and garlic. With great genetic diversity, the genus consists of more than 971 species that are described morphologically (Hauenschild et al. 2017) having great mixed-ploidy species in this monocotyledon genus (Peruzzi et al. 2017). For introgression of tolerant or resistant alien genes from wild or relative species into the domesticated commercial cultivars/species for various abiotic stresses in allium, interspecific or wide hybridization could play a great role. Not much work has been attempted in Alliums regarding abiotic stress response (Khandagale et al. 2020). In this genus, some efforts for the wide or interspecific hybridization have been attempted (Keller et al. 1996; Peffley and Hou 2000) but not in garlic owing to its sterility and lack of sexual reproduction. Studies on introgression of fertility and disease resistance genes through interspecific hybridization into cultivated garlic by using leek (*Allium ampeloprasum* L.) and garlic (Sugimoto et al. 1991; Yanagino et al. 2003) are reported. Leek is a leafy vegetable and seed propagated allium crop and might have the probability to form a bulb during hot season. Garlic is a bulbous crop and asexually propagated. For the creation of genetic diversity with specific traits, interspecific hybridization among garlic and leek might be the best approach for the climate-smart cultivar development in Alliums. Yanagino et al. (2003) successfully produced interspecific hybrids among garlic and leek by culturing ovary. They used some identified fertile clones of garlic as male parent through ovary culture. The hybridity of the interspecific cross was validated through morphological observations cytologically ($2n = 3x = 24$) and molecular analysis using random amplified polymorphic DNA (RAPD) markers. Further, their results revealed that the hybrid exhibited intermediate characteristics between the parental species such as growth, foliage, and bigger bulb size and garlic odor. Success and results of this interspecific hybrid indicated that this could have the potential to be a new crop having diverse genetic makeup and wide adaptability under changing climate conditions.

Some allium species including *A. vavilovii, A. roylei, A. altaicum, A. galanthum, A. fistulosum, A. oschaninii and A. pskemense* have been documented as the most important gene pools for the genetic improvement of onion for quality, biotic and abiotic stresses (Shigyo and Kik 2008). Leaf waxiness is a morphological trait that can protect plants from abiotic stresses especially heat stress (Mondal et al. 2015). Since long, wide hybridization in Alliums has been exploited for generating genetic variability, introgression of desirable genes and traits in the cultivated cultivars from wild species (Brewster 1994; Kik 2002; Singh and Khar 2021). Among wild species, *A. fistulosum* is used extensively for the interspecific crossing in Allium genus especially for onion genetic improvement and success could be increased through embryo-rescue technique (Dolezel et al. 1980). The first interspecific cross between these two species was reported by Emsweller and Jones (1935). The production of F1 hybrids between these two species could be easy, but with low pollen fertility (van der Valk et al. 1991). Khrustaleva and Kik (1998) introgressed genes from *A. fistulosum* to onion using a wild species *A. roylei* as a bridge species for more successful crossing.

7.7 Biotechnological and Genomic Approaches

Being a global phenomenon, changing climate is a constant limitation to all agricultural crops throughout the world. It has a long-term influence on overall agriculture in terms of crop productivity and ultimately food security. To address such undesirable variations and fluctuations is becoming a huge challenge for farmers and breeders (Raza et al. 2019). Thus, it is well predicted that future agriculture evolution will be designed by its response to climate change (Zilberman et al. 2018). Widely adapted climate-smart cultivars across different climatic conditions, various modern omics technologies along with conventional breeding approaches are required to combat various complex abiotic stresses.

In alliums, biennial nature, cross-pollination, inbreeding depression in onion; lack of flowering, large genome size in onion and garlic are major limitations for conventional breeding. Therefore, not much biotechnological and genomic work in Allium crops have been attempted (Shigyo et al. 2018) compared to other horticultural and field crops. Globally, few research groups have now started imparting focus and attention on Allium omics such as genomics, proteomics, transcriptomics, metabolomics, and metagenomics. With these approaches, we can speed up the development of climate smart cultivars using locally developed cultivars and landraces possessing climate-smart genes for the various stresses. With the advent of various biotechnological approaches like in vitro culture, genetic engineering, genomic selection, genome editing, improvement of Alliums could be started through using non-conventional approaches along with traditional breeding strategies. Figure 7.4 displays schematic representation of integrated 'omics' analyses in Allium crops (Adopted from Khandagale et al. 2020).

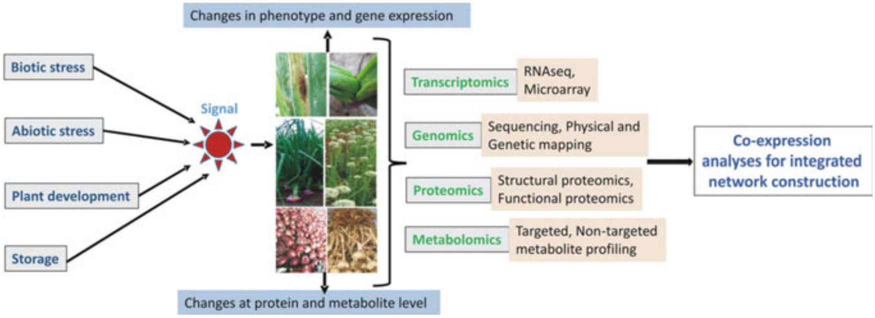

Fig. 7.4 Schematic representation of integrated 'omics' analyses in Allium crops (Adapted from Khandagale et al. 2020)

7.7.1 *In Vitro Selection for Abiotic Stresses*

Higher salt amounts result in reduction of protein content in the plant cells owing to elimination of K ions by the roots. K^+ ions are necessary for protein synthesis and its ions elimination triggers a physiological imbalance (Chen et al. 2007). Further, less potassium also deters growth and development of plants. Under prolonged salt stress, synthesis of proteins is significantly and negatively affected (Caplan et al. 1990). Under salt stress, proline amounts are enhanced which might be attributed to the fact that this functions as osmoprotectant in plants (Hayat et al. 2012; Liang et al. 2013; Singh and Wani 2019). In Egypt, Bekheet et al. (2006) exploited in vitro techniques to screen onion callus under various concentrations of salts. They observed decline in growth and fresh weight with enhancement of salts in culture media, but dry matter and weight was enhanced with higher salt concentration upto 4000 ppm then onwards suppressed. This study proved that onion cells are adapted to salt stress upto such limits. And the seedlings obtained can be a potential source for further onion improvement and breeding programs for abiotic stress tolerance. Consequently, in garlic, AI-Safadl and Faoury (2004) evaluated 25 garlic cultivars against salt stress using different five culture media with different salt concentrations. Lower amounts of salts gave stimulating effects while higher concentrations caused no growth to significant decline in shoot growth. Similarly, significant effects of salt on root growth were noticed to be genotypic specific. Among 25 cultivars under the study, two cultivars namely Kisswany and Hungary were found to be the most salt tolerant genotypes, both possessing 50% higher average shoot length than control under stress conditions. They concluded that cultivars producing small in vitro shoots under salt stress develop more or longer roots to compensate for the insufficiency of roots. According to the in vitro experiments conducted by Plabon et al. (2021) in Bangladesh, they determined that the onion cultivars showed no significant adverse effects upto 75 mM salt stress level under in vitro conditions. They found one genotype namely Faridpuri which could tolerate and perform well upto 100 mM salinity level.

7.7.2 Transcriptomic Analysis

Plants respond during water stress especially during drought in the form of changing pattern of the gene expression, metabolites like ABA accumulation and synthesis of specific proteins (Ghodke et al. 2020). Use of transcriptome analyses along with microarray technique and conventional breeding approaches have isolated various drought stress-responsive transcription factors (TFs) in different plant species (Vinocur and Altman 2005; Bartels and Sunkar 2005). No systematic transcriptomic study has been conducted in alliums for abiotic stresses. Ghodke et al. (2020) studied drought response through transcriptome approach in onion. They sequenced transcriptomes of two contrasting drought sensitive onion genotypes (drought-tolerant Acc#1656 and drought-susceptible Acc#1627) under drought stress through Illumina paired-end sequencing technology. Differential gene expression analyses elicited downregulation and upregulation of 1180 and 1189 genes, respectively, in Acc#1656. In Acc#1627, downregulation and upregulation of 1292 and 872 genes, respectively, was recorded. Genes encoding transcription factors, membrane transporters, cytochrome P450, flavonoids, and carbohydrate metabolism exhibited differential expression in the susceptible and tolerant onion genotypes.

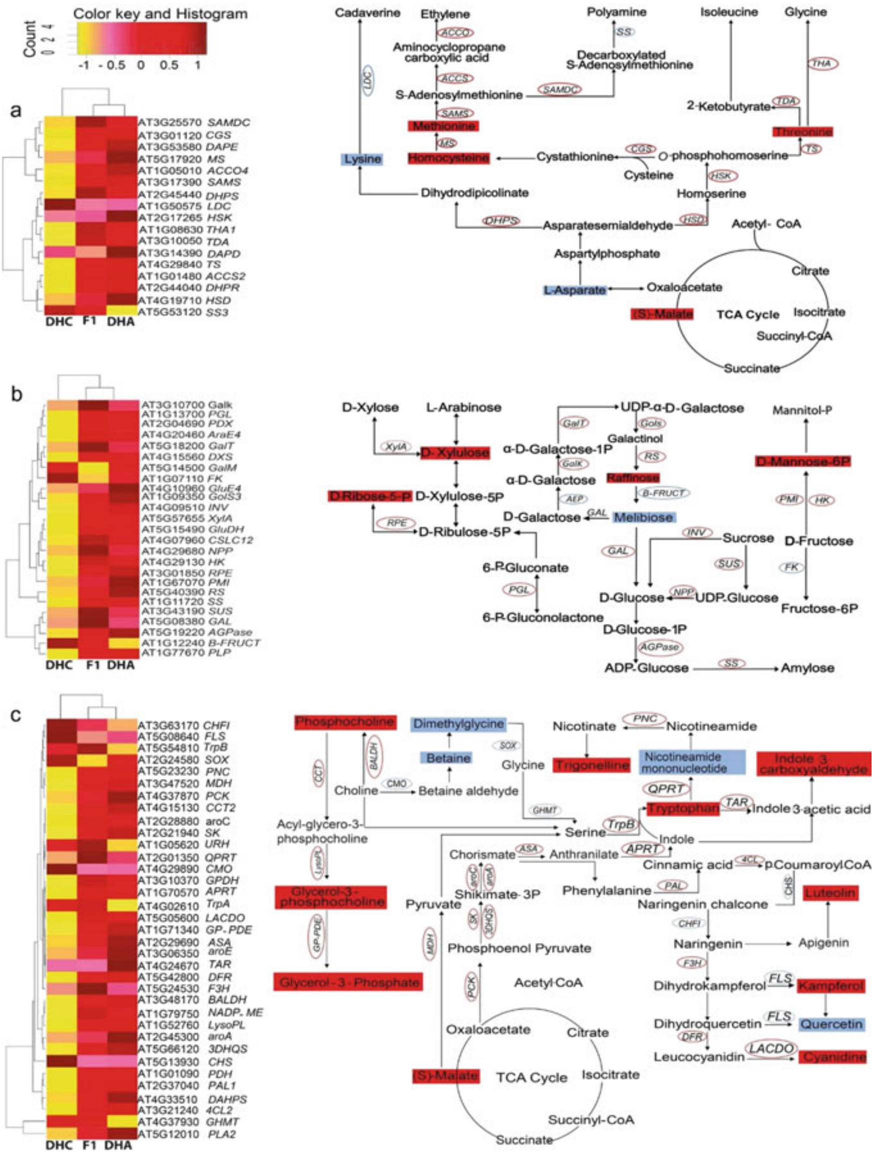

Fig. 7.5 Model summarizing metabolites change and gene expression analysis associated with amino acid biosynthesis pathway (**a**), carbohydrate biosynthesis pathway (**b**) and shikimate, flavonoid, tryptophan, and phospholipid biosynthesis pathway (**c**) in the DHC, DHA and F1. Metabolites and genes with significant accumulation ($P < 0.05$) in DHA and F1 highlighted with red color and DHC with blue (Adapted from Abdelrahman et al. 2015)

In Japan, scientists produced F1 hybrid by crossing tropical (shallot doubled haploid *A. cepa* L. Aggregatum group, DHA) and cultivated onion (doubled haploid *Allium cepa* L., DHC) species having genetic and metabolic parental properties that could be quite beneficial to study abiotic stress tolerance in Allium. The major genes and metabolites were localized through targeted transcriptome and metabolome profiling of onion under normal and stressed conditions. Among 113 targeted metabolites, about forty-nine were observed significantly different between genotypes (Abdelrahman et al. 2015). Model summarizing metabolites change and gene expression analysis associated with amino acid, carbohydrate, shikimate, flavonoid, tryptophan and phospholipid biosynthesis pathways in the DHC, DHA and F1 have been presented in Figs. 7.4 and 7.5.

Thermal stress such as low temperature or cold (0–15 °C) and freezing (less than 0 °C) stresses are the common abiotic stress which adversely and significantly affect the plant production due to various manipulations in biochemical and physiological activities as defense systems for enduring such thermal stresses. In the winter season, cold or low temperature can be a great challenge for the bulb onion production. In onion, Han et al. (2016) applied high-throughput sequencing technology to low temperature treated and control samples of cold susceptible and tolerant genotypes. A total of 452 million paired-end reads were de novo assembled into 54,047 genes with an average length of 1331 bp. Among 5167 DEGs, 491 genes were differentially expressed at freezing compared to control temperature in both susceptible and tolerant libraries. Sufficient number of molecular markers identified from the assembled genes, included 4437 SSRs and SNPs substitutions of transition and transversion types of both the genotypes.

7.7.3 Genome-Wide Association Studies (GWAS) for Stress Tolerance

To understand the full set of genetic variants in crop cultivars and to identify allelic variants linked with any specific trait, genome-wide association studies (GWAS) is one of the powerful genomic technologies (Manolio 2010). GWAS has been conducted to reveal the genetic background responsible for the resistance at the genetic level under climate change (Mousavi-Derazmahalleh et al. 2018). In plants, GWAS has widespread applications related to biotic and abiotic stresses (Lafarge et al. 2017; Thoen et al. 2017). The first work of high-throughput garlic genotyping was done by Egea et al. in 2017. They reduced significantly garlic germplasm bank size, identifying redundant accessions and thus generating a unique (non-redundant) core collection, with the consequent reduction in space and maintenance expenses. They further suggested that DArTseq analysis is a cheaper method to perform genotyping-by-sequencing and genetic diversity analyses of garlic having gigantic complex and without a reference genome, and gave reliable results, according to genotype and their geographical origin. With this study, it would be easy for the breeders to select a genotype from a characterized core collection for better adaptability against various biotic and abiotic stresses under changing climate and global warming.

7.8 Future Perspectives

Allium species are the important commercial horticultural crops, owing to their health promoting and nutraceutical properties. Besides the fresh consumption of onion and garlic, processing of these crops is a great venture as a processing industry. With the recent developments and awareness among the consumers, demand of onion and garlic is surging. Like other crops, allium production is also threatened and challenged by various biotic and abiotic stresses under a rapidly changing climate scenario. Among abiotic stresses, thermal (high and low temperature), moisture (waterlogging and drought), salinity and heavy metals are the critical and prominent stresses for the alliums. For sustainable allium production, creation of genetic variations through breeding and exploiting other management methods to alleviate these stresses is imperative. In garlic, breeding is restricted due to lack of sexual reproduction and sterility. Nevertheless, in case of onion, it is easy to develop genetic variations through breeding approaches to develop climate smart cultivars. Presently, genetic advancement in garlic has been achieved through clonal selection. For more genetic variation, it needs utmost attention to develop and standardize in vitro biotechnological protocols such as somaclonal variations, in vitro mutagenesis, gene or genome editing through CRISPR/Cas9 technology. However, exploitation of omics technology in allium is limited due to its huge genome size, biennial life cycle, highly cross-pollinated nature and high inbreeding depression. The biggest challenge in garlic is to restore fertility, which will create new possibilities for its genetic improvement through conventional and modern breeding approaches. Being so complicated physiologically and genetically, abiotic stress tolerance development in alliums is a very cumbersome and tedious task as meagre research work has been carried out systematically. In spite of biosafety issues, genetic transformation could be another option to mitigate abiotic stresses through *Agrobacterium* or biolistic approach of desired gene transfer. It is very clear that there are still big allium research and knowledge gaps at genetic and molecular level at present. Very few validated molecular markers are available in alliums; only RAPD, SSR have been developed for their diversity analysis. In alliums, there is a need to pay more attention on the root system and root architecture to mitigate various abiotic stresses such as salinity and drought. Exploitation of osmoprotectants like proline, α-tocopherol, glycine betaine, etc. might have been better option as abiotic stress management strategy in alliums. Being the largest monocot, Allium genus possesses more than 900 species, screening of various cultivated, wild and related species from different geographical locations for various abiotic stress tolerance might be stressed in a collaborative mode. Furthermore, proper understanding of the abiotic stress tolerance mechanism at molecular, physiologically and biochemically in alliums in terms of changing climatic patterns needs more focus and attention of the breeders and physiologists in future for the development of contingency approaches for the genetically designing of climate resilient genotypes or cultivars for sustainable allium production.

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Chapter 8
Genomic Designing for Improved Abiotic Tolerance in Amaranth: An Integrated Approach of Genetic Diversity and Tolerance Phenotyping

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Abstract Amaranth (*Amaranthus* spp.) is an underutilized C_4 vegetable and grain crop which grows successfully in low input environments and has diverse abiotic stress tolerance traits. In addition to being highly nutritious, it is genetically diverse and phenotypically plastic, making it a suitable crop for nutritional security in a rapidly changing climate. Drought and salinity are two important environmental factors affecting productivity worldwide. A deeper understanding of stress tolerance mechanism in amaranth is crucial especially when stress tolerance characteristics are governed by many genes. Moreover, improvement of crop plants relies on a large pool of genetically diverse material available. Genebanks hold large collections of untapped resources but there is a cost to maintain large number of accessions and it can be difficult to discern which traits are of value. Precise germplasm phenotyping for abiotic stress tolerance is therefore an effective way of selecting materials for breeding. This requires affordable, rapid and efficient trait phenotyping technique. With the availability of the high-quality reference genome sequence of grain amaranth *(Amaranthus hypochondriacus),* new targets of selection become more feasible through genome-wide data. A genomic approach through molecular tools such as next generation sequencing (NGS) and DNA markers enable mapping of any traits that can be measured and generate significant data on the genetic control of traits and their interaction with the environment. The genetic potential of a crop depends on the plant phenotype, which is a result of the interactions between the genome and the environments. This chapter discusses the fundamentals of amaranth responses to

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abiotic stress for precision phenotyping, a vital step before implementing genomic approach for improvement of abiotic tolerance in crop plants. The chapter looks at the crop's past and present research on genetic diversity and the prospects of integration of genomic approach and plant physiology in the development of superior amaranth cultivars.

Keywords Amaranth · C_4 pseudo cereal crops · Abiotic stress · Drought tolerance · Salt tolerance · Germplasm phenotyping · Amaranth genome

8.1 Introduction

The genus *Amaranthus* consist of 60–70 diverse and interesting species, comprising of cultivated pseudocereal crops (*A. caudatus*, *A. cruentus* and *A. hypochondriacus*), vegetable crops (*A. blitum and A. tricolor*) and the world's worst weeds (*A. palmeri, A. virdis, A. retroflexus, and A. hybridus*) (Das 2012). Amaranth belongs to the Amaranthaceae family along with other economically important crop in the family, *Chenopodium* (quinoa and canahua), *Beta* (beet and sugar beet) and *Spinacia* (spinach). Amaranth is a C_4 dicotyledonous plant (Kauffman and Weber 1990), possesses many desirable agronomic traits including a unique nutritional profile both in grain and leaves (De Ron et al. 2017; Jiménez-Aguilar and Grusak 2017), substantial amount of genetic diversity (Brenner et al. 2000; Stetter et al. 2017), tolerance to abiotic stress (Barrio and Anon 2010), high phenotypic plasticity (Khanam and Oba 2014) and can survive in highly contrasting habitats, from arid and semi-arid zones to saline soil conditions (Huerta-Ocampo et al. 2014; Saucedo et al. 2017; Sarker et al. 2018). Partly as the consequences of its C_4 expressions, amaranth can maintain carbon assimilation by maximizing water use efficiency (WUE) during drought and salinity stress (Liu and Stützel 2002a, b, 2004; Slabbert and Krüger 2011; Jamalluddin et al. 2019). In the context of diversifying food basket from over-reliance on maize (*Zea mays*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) in an increasingly uncertain world, amaranth offers an alternative nutritious diet for future food and nutrition security.

Historically, grain amaranth was one of the oldest crops in Americas, cultivated as a sacred staple crop with an equal importance status with corn and bean (*Phaseolus vulgaris*) during the Aztec civilizations, and consumed by all social classes 5000–7000 years ago (Sauer 1950; Caselato-Sousa and Amaya-Farfán 2012). However, amaranth cultivation was suppressed during Spanish colonization due to its strong spiritual belief (Iturbide and Gispert 1994). The dormant popularity of amaranth was revived by Robert Rodale from rediscovery of sacred Aztecs' crops by Sauer (1950) and Downton (1972), and after a decade of intense research involving worldwide farmers, researchers and advocates (Lehmann 1996), amaranth was declared "The Future Food" by the United States in the 1980s (National Research Council, Amaranth: Modern Prospects for an Ancient Crop, National Academy Press, Washington, DC 1986). Currently, it has worldwide distribution, but most species predominate in the warm temperate and tropical regions (Parra-Cota et al. 2014). However, as of today, the status of amaranth remains as an underutilized or orphan crop, indigenous crop species which are well-adapted to marginal lands and typically have low input needs, restricted to a local production and consumption system (Mayes et al. 2011; Massawe et al. 2016; Mabhaudhi et al. 2016).

A paradigm shift in food production systems toward sustainable diets allows utilization of a wide range of crop species that can cope with dramatic climatic variations (Chivenge et al. 2015; Mustafa et al. 2019). There is a global trend for the increasing frequency and severity of droughts (Dai 2013) which are expected to increase in the next 30–90 years (Wilhite 2005). Soil salinity is also predicted to increase through a rise in sea levels, and by 2050, 50% of cultivated land may be lost through soil salinization (Flowers et al. 2010). Drought and salinity are the most important environmental factors that hamper the yield loss worldwide. The International Disaster Database recorded 642 drought events from 1900 to 2013 in Africa, The Americas, Asia, Europe and Ocenia, affecting over 2 billion people (Masih et al. 2014). This will affect more than 1.1 billion people in South Asia and Sub-Saharan Africa who are relied on agricultural sector and additionally, 75% of these people live in poverty (Vermeulen et al. 2012; Ali et al. 2017; Twongyirwe et al. 2019). Both stresses can induce several morphological, physiological, biochemical and metabolic alterations (Kumar et al. 2012; Chatterjee and Solankey 2015; Fang and Xiong 2015; Hanin et al. 2016; Lamaoui et al. 2018; Isayenkov and Maathuis 2019; Van Zelm et al. 2020). Most plants use more than one strategy at a time to resist stress (Mitra 2001), and different traits are required to mitigate different types, severity and duration of stress (Kamoshita et al. 2008), dependent on plant species and genotypes, and plant age and size (Ma et al. 2020). Therefore, deeper understanding on the genetic and phenotypic variations in amaranth in response to abiotic stress is crucial as it often involves complex polygenic traits. For a successful integrated breeding, large sets of informative phenotypic data are needed (Cooper et al. 2014; Yugandhar et al. 2017). However, for orphan leafy vegetables, basic knowledge related to morphological and physiological traits for abiotic tolerance is still lacking (Sogbohossou et al. 2018; Sarker and Oba 2020). A development of rapid, affordable and efficient phenotyping technique must consider multiple factors that are involved in abiotic stress for breeding material selection. This is because phenotyping for stress tolerance requires larger space, time-consuming, expensive and lack of seed resources (Hura et al. 2007).

In the past two decades, remarkable progress in trait phenotyping for stress tolerance, and the integration of genomic platforms has accelerated the selection of resistant cultivars in major crops (Tuberosa et al. 2014) such as maize (Cooper et al. 2014) and rice (Kumar et al. 2014). The genetic potential of a crop depends on the plant phenotype, which is a result of the interactions between the genome and the environments. A genomic study through molecular tools such as next generation sequencing (NGS) or DNA markers could generate significant data on the genetic control of traits associated with abiotic stress and this approach would give species- and trait- specific results, with a deep understanding of the phylogenetic relationships of those crops (Mayes et al. 2011). More recently, the substantial advances in understanding genome systems and the plummeting cost of sequencing have driven genotyping not only to non-model crops, but to individual cultivated landraces (Elshire et al. 2011; Andrews et al. 2016). Whole genome sequencing can facilitate the fast development of tolerant cultivars by assessing diversity in ex-situ collections to map quantitative trait loci (QTLs) for candidate genes (Varshney et al. 2013), allow comparative genomics to understand synteny and functional genes with closely related species (Hardison 2003), and facilitate genomic selection in predicting genotype performance (Shikha et al. 2017). This chapter discusses the fundamentals of amaranth responses under abiotic stress, focussing on the most affecting stresses, drought and salinity for precision phenotyping, a vital step before implementing genomic strategies. It looks at the crop's genetic and phenotypic diversity and the prospects of integration of genomic approach and plant physiology in development of superior amaranth cultivar.

8.2 Understanding the Functional Basis of Abiotic Stress Mechanisms in Amaranth

8.2.1 Prospects of C4 Photosynthesis: Leaf Anatomy, C4 NAD-ME Subtype and Limiting Factor of Photosynthetic Water-Use Efficiency

The C_4 plants are historically grouped into three distinct biochemical pathways known as enzyme of malate metabolism, the C_4 acid decarboxylation enzyme in the bundle sheath: NAD-dependent malic enzyme (NAD-ME), NADP-dependent malic enzyme (NADP-ME) and PEP carboxykinase (PEPCK) pathway (Hatch 1987; Hattersly 1992). However, no pure PEPCK-type has been discovered in any C_4 species (Sage 2004) and PEPCK pathway exists in multiple lineages across different genus and therefore, only NAD-ME or NADP-ME subtypes are currently known as distinct C_4 biochemical pathways with or without the additional service of the PEPCK pathway (Wang et al. 2014). Amaranth belongs to the NAD-ME subtype of C4 plants (Ueno 2001; Babayev et al. 2014), together with switchgrass (*Panicum virgatum* L.) and pearl millet [*Pennisetum glaucum* (L.) R. Br], which use NAD^+ as a cofactor during decarboxylation. Many other cereals belong to the NADP-ME subtype, which use NADP + as a cofactor, including maize, sugarcane (*Saccharum* spp.), and sorghum (*Sorghum bicolor*) (Edwards and Walker 1983). NAD-ME acts primarily as an antioxidant in catabolism which produces energy (ATP) through oxidation (Chen et al. 2019). The enzyme activity and structure of NAD-ME subtypes are unique to species, either in monocot or dicot plants, for instance, large heterodimeric photosynthetic NAD-ME α and β subunit encoded gene are found abundantly in mitochondria of the dicot *A. hypochondriacus* (Long et al. 1994), meanwhile only NAD-ME β

subunit encoded gene is found in the bundle sheath cells of monocot switchgrass (Rao and Dixon 2016).

NAD-ME and NADP-ME may play different roles in the metabolism and synthesis of substances, including responses to light dependent reactions (Rao and Dixon 2016; Sun et al. 2019) and these may be due to their specific characteristics in leaf anatomies and organelles (Hattersley 1992; Yoshimura et al. 2004). However, in some plants, the expression of NAD-ME and NADP-ME subtypes may be associated with the plant species phylogeny and the C_4 subtype lineage, within which both malate metabolic pathways may be present together at some level (Washburn et al. 2015). Liu and Osborne (2015) reported that the interaction of phylogenetic lineage, NAD-ME subtype and adaptation to habitat water ability were strongly influenced the tolerance of plants. The phylogeny lineage tended to be associated with leaf structural traits while NAD-ME subtype tended to be more related to physiological traits such as instantaneous response like stomatal behavior and enzyme activity. The NAD-ME subtype occurs more frequently in dry areas (Taub and Lerdau 2000), had longer and narrower leaves with smaller and denser stomata, thus exhibit superior water use efficiency under drought conditions (Ghannoum 2009) compared to the NADP-ME subtype (Liu and Osborne 2015). However, which traits regulate the photosynthetic capacity of amaranth remains unclear. Nonetheless, *A. hypochondriacus* and wild amaranth species including *A. hybridus*, *A. powelli* and *A. retroflexus* have been shown to have high photosynthetic rate under drought conditions, probably due to their unique leaf Kranz anatomy and the NAD-ME pathway that concentrates CO_2 at Rubisco, thus suppress photorespiration (El-Sharkawy 2016). Meanwhile, high salt tolerant *A. caudatus* was shown to be associated with reduced stomatal density and stomatal conductance which contribute to a lesser water loss under salt stress (Estrada et al. 2021). This eco-physiological adaptation may be associated with its leaf structure and NAD-ME subtype, which results in higher resistance to drought and salt conditions.

Amaranth possesses C_4 Kranz anatomy (El-Ghamery et al. 2017). The quantitative balance of well-developed grana and almost equal accumulation of starch in both bundle sheath and mesophyll cells of amaranth (Hattersley and Watson 1992; El-Ghamery et al. 2017) are critical to regulate photosynthesis (Ueno et al. 2006; Joaquín-Ramos et al. 2014). It also able to maintain the plant growth of *A. tricolor* under salt stress by developing more grana to make up for the loss of PSII activity in the mesophyll cells when salinity stress is too severe (Omoto et al. 2010). Increased severity of salt stress causes grana in bundle sheath cells of *A. cruentus* became elongated and increase the number of plastoglobules (Joaquín-Ramos et al. 2014), and reduced mesophyll size in *A. caudatus* (Estrada et al. 2021). In drought conditions, a new isoform of NAD-ME enzyme has been found in the mitochondrial fraction of bundle sheath cells of *A. cruentus* which then disappears upon re-watering (Babayev et al. 2014). The isoform contributes to the accumulation of CO_2 supplies during drought stress, indicating its potential role in drought adaptation (Babayev et al. 2014). An accumulation of drought and salinity stress responsive proteins have been observed in *A. cruentus* and *A. hypochondriacus* including chloroplast chaperonins that involves in refolding and protein complexes protection (Huerta-Ocampo et al. 2009; Joaquín-Ramos et al. 2014). This demonstrates that the chloroplast and mitochondria play an important role in cell adaptation and restoration in amaranth during abiotic stress. Moreover, Rubisco may be the rate limiting enzyme for photosynthesis in amaranth (von Caemmerer and Furbank 2016; Tsutsumi et al. 2017). Drought stress has also caused downregulation of the Rubisco large subunit, reducing the carbon metabolism (Huerta-Ocampo et al. 2009). Joaquín-Ramos et al. (2014) also reported that Rubisco decreased in both bundle sheath and mesophyll cells under severe salinity stress in *A. cruentus*. The regulation of Rubisco expression in amaranth is complex dependent on the age and plant size, species, and variety, in which Rubisco is expressed as a C_3-type pattern when leaves are young and only expressed as a C_4-type pattern as the leaves expand (Cormac et al. 1997). These suggest that some biochemical or physiological traits involved in photosynthesis under normal and stress conditions, whereas structural traits are not directly involved.

8.2.2 Betacyanin and Other Natural Antioxidants in Amaranth: Regulating Osmotic Adjustment and Osmoprotective Against Reactive Oxygen Species (ROS)

In general, a plant resistance to stress depends on its protective mechanism and restoration of damage capabilities. Stress induces the activation of reactive oxygen species (ROS) which include superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) (Mittler 2002; Neill et al. 2002). The ROSs are highly reactive, they can disrupt normal metabolism through oxidative damage to organelles particularly photosynthetic apparatus, lipids, protein and nucleic acids (Rout and Shaw 2001), and can generate photooxidation stress (Wang et al. 2013). In amaranth, antioxidative system are highly stimulated under drought stress, such as an increased of superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) (Slabbert and Krüger 2014), similar to maize (Köşkeroğlu and Tuna 2010). The combined effects of these antioxidant enzymes may neutralize ROSs activities during oxidative stress. The increased activities of SOD, APX and catalase (CAT) have been correlated with proline accumulation in amaranth during drought and salt stress (Slabbert and Krüger 2014; Sharma et al. 2019). This suggests that the antioxidant defence mechanism is activated by the increase of proline accumulation (Yan et al. 2000; Ahmed et al. 2009).

Proline is α-amino acid normally accumulate in the cytosol to regulate cytoplasmic osmotic adjustment (Ashraf and Foolad 2007; Marek et al. 2009; Zadehbagheri et al. 2014), membrane stabilization (Hayat et al. 2012) and a precursor of antioxidizing enzymes (de Carvalho et al. 2013) during drought stress and decreases rapidly upon re-watering (Hare et al. 1998). Besides, high proline content is associated with drought tolerance in some crops, including cotton (Zhang et al. 2014) and chickpea (*Cicer arietinum* L.) (Ghiabi et al. 2013), sunflower (Umar and Siddiqui

2018) and faba beans (El-Harty 2016). As shown by Umar and Siddiqui (2018), high number of leaves, leaf area, RWC and plant height under drought stress could be due to the higher accumulation of proline. High accumulation of proline may influence the activation of antioxidizing enzymes that scavenge ROS during drought stress, as observed in a study conducted by Slabbert and Krüger (2011) in *A. tricolor* genotypes. This suggests that the increase of proline production during drought stress may activate antioxidative defence mechanism (Yan et al. 2000; Ahmed et al. 2009) and thus, reduce the oxidative damage to plants' organelles (Rout and Shaw 2001). It was also reported that proline may sometimes have a slight quantitative contribution to osmotic adjustment (OA), but its major effect is in stabilizing cellular structures (Sánchez et al. 1998; Shabala and Shabala 2011) and cell wall modification (Maatallah et al. 2010).

Amaranthine is the main betacyanin pigment in amaranth that contributes to the red or purple colour of the plants. It had been reported that amaranthine possesses high ROS scavenging activity during drought and salinity in amaranth (Sarker et al. 2018) due to its abundance of hydroxyl and imino groups (Cai et al. 1998; Strack et al. 2003). Red (betacyanic) and green (acyanic) leaves of amaranth have similar chlorophyll content and chlorophyll a/b ratio, hence both have similar light-harvesting capacity (Nakashima et al. 2011). However, while it has been observed that relative water content (RWC), photosynthesis and chlorophyll content are equally reduced under drought stress, photoinhibition is severe in green leaves compares to red. Higher betacyanin pigments in red amaranth than the green possibly the reason of better adaptation to drought and salinity stress as it has stronger radical quenching capacity (Shao et al. 2013), thus increased the total photoprotective capacity (Nakashima et al. 2011). The increased pigment accumulation does not coincide with betacyanin precursor activity, dihydroxyphenylalanine oxidation tyrosinase (DOT) hydroxylation of tyrosine, hence DOT activity may not be necessarily required under certain circumstances such as water stress (Casique-Arroyo et al. 2014).

The RWC is an indicator of leaf water status during drought and salinity periods (Blum 1998; Uzildaya et al. 2012; Sarker and Oba 2020). *A. tricolor* has been shown to have a high RWC (77%) under severe drought compared to other amaranth species, *A. hybridus* (48%) and *A. hypochondriacus* (33%) (Slabbert and Krüger 2014). Besides, RWC has been successfully used as a screening tool for selecting drought tolerant cultivar in other crops such as potato (Soltys-Kalina et al. 2016), cowpea (Zegaoui et al. 2018) and beans (Rosales et al. 2011). RWC is an accurate assessment for osmotic adjustment in plants (Sanders and Arndt 2012; Blum 2016). OA is an indicator of plant survival through cell turgor or stomatal conductance and has also been recognized as a prime adaptive trait for higher yield under drought stress across diverse crops (Blum 2016). High RWC was associated with high OA in some crops such as castor bean (*Ricinus communis* L.) (Babita et al. 2010) and sunflower (*Helianthus annuus* L.) (Rauf and Sadaqat 2008). OA is regulated through accumulation of organic solutes (such as proline, glycine betaine and total soluble sugar) or inorganic ions (such as K^+ and Ca^{2+}). These solutes may be obtained from inorganic salts in soil and from products of photosynthesis which helps to protect cellular proteins, enzymes, and cellular membrane against cell dehydration (Zivcak et al. 2016). Proline accumulation is considered as a general marker of drought tolerance (Liu et al. 2011) as it permits OA, and negative correlation has been found between OA and RWC under drought stress in faba beans (*Vicia Faba* L.) (El-Harty et al. 2016) and ornamental shrubs (*Eugenia uniflora* L.) (Toscano et al. 2016). This demonstrates that the synthesis of proline increases as RWC declines.

8.3 Germplasm Evaluation in Amaranth: Unveiling Taxonomy Classification and Genotypic Variations in Stress Response

Germplasm phenotyping is one way to identify amaranth accession with superior stress tolerance traits (Passioura 2012; Mwadzingeni et al. 2016). Knowledge of genetic diversity and trait variations in crop germplasm is important for plant breeding and for developing plant genetic resources with improved traits (Akin-Idowu et al. 2016). Amaranth has a large amount of genetic diversity, and this may provide the opportunity to select better performing genotypes for any trait of interest (Savita 2006). To achieve this, germplasm must be accurately assessed and evaluated to improve the genetic resources of commercially important lines. The assessment of diversity is routinely performed using various markers, including morphological, biochemical, and molecular markers (Govindaraj et al. 2015). A high-quality core set can characterize and capture maximum genetic diversity of the entire germplasm collection and resolve redundancy problems (Frankel and Brown 1984; Liu et al. 2015).

Multiple domestication, speciation and cross hybridization in *Amaranthus* genus made it difficult to establish the exact species number and taxonomy of the whole genus (Costea et al. 2004; Wassom and Tranel 2005; Judd et al. 2008). It is possible that only a fraction of available accessions is accounted for species number despite many amaranth hybrids (Jacobsen and Mujica 2003). Phenotypic and genetic markers have been used to investigate the taxonomic and phylogenic classification of amaranth, however, none of the published studies can identify a consistent classification (Lanoue et al. 1996; Chan and Sun 1997; Wassom and Tranel 2005; Das 2012; Jimenez et al. 2013; Stetter and Schmid 2017). Until recently, the use of single nucleotide polymorphisms (SNPs) discovery through genotyping-by-sequencing (GBS) in amaranth is the most efficient method to resolve taxonomy and evolution of the genus, and to validate amaranth phylogeny with consistent geographical origin and morphological classification (Stetter et al. 2017; Wu and Blair 2017).

8.3.1 Phenotype-Based Diversity Analysis

Traditionally, the primary sources of genetic diversity are identified through variation in morphological traits. It provides useful information on the diversity patterns within and among populations (Veasey et al. 2008), and agronomic traits of interest can be identified through naked eye observation and certainly expressed under different climatic conditions (Ahmad et al. 2018). The morphological characteristics such as leaf color and plant height can be used as a direct assessment for material selection (Krichen et al. 2012). Qualitative traits such as leaf, stem and petiole colour and shape are chosen for consumer's preferences (Akaneme and Ani 2013), capable of classifying the genus *Amaranthus* into amaranth species (Gerrano et al. 2014) and have been used as quality traits for drought tolerance characteristics (Nakashima et al. 2011). In leafy vegetable amaranth breeding, leaf yield is the primary target trait with high heritability and genetic advances have been estimated in *A. tricolor*. A strong correlation between leaf yield with plant height, number of leaves and stem diameter has been observed in amaranth (Shukla et al. 2006; Sarker et al. 2014; Sogbohossou et al. 2018). Therefore, leaf yield could be significantly improved through direct selection of these traits.

The floral parts and seed morphology have been used for taxonomic identification (Trucco and Tranel 2011). *Amaranthus* genus have been grouped into three sub-genera, which are *Amaranthus Amaranthus* (grain), *Amaranthus Albersia* (vegetable), *Amaranthus Acnida* (weed), which has been defined using seeds, inflorescence and floral characteristics (Mosyakin and Robertson 1996; Trucco and Tranel 2011; Das 2012; Achigan-Dako et al. 2014). Grain amaranth is characterized by a moderate to large complex apical inflorescence comprising aggregates of cymes, five tepal lobes and five stamens, variable seed coat colour and well-defined flange, utricle circumscissile (Das 2012). The dicotyledonous nature of amaranth species precluded them from being classified as a cereal, as true cereals are monocotyledonous grasses. Therefore, grain amaranth is referred to as a pseudo-cereal. Vegetable amaranth can be distinguished by its inflorescence and indeterminate growth habit, possession of axillary glomerules or short spikes, flower buds from the leaf axil, three tepal lobes and stamens, and has brownish black seed with undifferentiated folded flange.

Morphological data is still worthwhile for the development of a core set of large germplasm accessions (Upadhyaya 2003; Archak et al. 2016) and has been used to successfully characterise genetic variation in several amaranth species (Oboh 2007; Pandey 2009; Shukla et al. 2010; Selvan et al. 2013; Akhter et al. 2013; Sarker et al. 2015; Akin-Idowu et al. 2016; Gerrano et al. 2017). Despite of having well-defined characters, species classification based on morphological characters often been challenging in amaranth because of the huge phenotypic variations within and between species (Mandal and Dhangrah 2009), their complex genetic structure (Banerjee and Kole 2009) and broad geographical distribution (Mujica and Jacobsen 2003). These variations could be due to gene combination from polyploidy event (Andini et al. 2013), or mixed-mating system (Kulakow and Hauptli 1994), or because of recent domestication event (Chan and Sun 1997). Besides that, morphological descriptors are notoriously plastic due to environmental influence (Sauer 1967; Espitia 1992; Tabatabaei et al. 2011). Therefore, combined analysis using morphological and molecular markers is routinely performed to produce more accurate data on genetic, phenotypic and their environment interactions (Malviya et al. 2012).

8.3.2 Genetic-Based Diversity Analysis

Genotyping using molecular markers has been successfully applied to many crops for the development of population structure and genetic diversity (Cavanagh et al. 2013; Laidò et al. 2014; Nadeem et al. 2018), to validate phylogeny of the genus (Stetter et al. 2017), identify QTLs and candidate genes conferring valuable traits (Barilli et al. 2018) and generate data for gene expression profiling (Kouzai et al. 2016). Different types of molecular markers have been developed on *Amaranthus* genus for genetic diversity, genome evolutionary and taxonomic differentiation, including, random amplified polymorphic DNA (RAPD) (Transue et al. 1994; Mandal and Das 2002; Popa et al. 2010), amplified fragment length polymorphism (AFLP) (Xu and Sun 2001; Wassom and Tranel 2005; Costea et al. 2006; Oduwaye et al. 2014; Štefúnovà et al. 2014), inter simple sequence repeat (ISSR) (Raut et al. 2014); simple sequence repeat (SSR) (Lee et al. 2008; Mallory et al. 2008; Khaing et al. 2013; Oo and Park. 2013; Wang and Park 2013; Kietlinski et al. 2014; Suresh et al. 2014; Nguyen et al. 2019) and SNPs (Maughan et al. 2009; Jimenez et al. 2013; Stetter et al. 2016; Wu and Blair 2017), bacterial artificial chromosome library (Maughan et al. 2008), genetic maps (Maughan et al. 2011), transcriptome (Riggins et al. 2010; Delano-Frier et al. 2011; Liu et al. 2014; Sunil et al. 2014).

The majority of markers listed above have been useful for evolutionary, phylogenetic and genetic diversity although limited number of markers were being utilized and did not cover the whole genome (Mallory et al. 2008; Maughan et al. 2009, 2011; Kietlinski et al. 2014). However, the markers unable to distinguish the amaranth accessions into specific geographical origin and morphological stratification (Jimenez et al. 2013). Nonetheless, the recent used of high-density SNPs from GBS data can classify amaranth into consistent geographical origin and has proved that comprehensive origin sampling can assist in understanding the evolution of the genus (Wu and Blair 2017). This is shown by a strong split geographic pattern in *A. hybridus* from Central and South America, which later confirmed that these two different lineages were the ancestors of the grain amaranth (Stetter and Schimdt, 2017).

An admixed population structure in amaranth indicated that frequent hybridization or introgression events had happened and thus produced new gene combinations (Lee et al. 2008). This is probably due to the cosmopolitan nature of the genus, or the results of human activities such as breeding and resources exchange (Lee et al. 2008). An experiment based on SSR markers by Khaing et al. (2013) revealed that *A. tricolor* scattered to different groups which may imply that *A. tricolor* had large genetic variations. There was also uncertainty in positioning phylogeny of *A. tricolor* accessions among amaranth species, although *A. tricolor* accessions were grouped together in a clade (Stetter and Schmid 2017). *A. tricolor* has a significantly larger estimated genome size among 35 amaranth species, and this suggests that polyploidization likely influenced the genome size of this species (782.7 Mbp) (Stetter and Schmid 2017). In weedy amaranth, *A. palmeri* and *A. retroflexus*, the varying amount of crossing resulting in subsequent gene flow between populations and may occur more rapidly than the primarily self-pollinated amaranth species (Stetter et al. 2016), and this can shape adaptive evolution and become more invasive (Kistner and Hatfield 2018). While amaranth genotyping using markers have been useful in evolutionary, phylogenetic and germplasm characterization, marker validation is still needed to facilitate amaranth breeding with increased abiotic stress (Wu and Blair 2017).

8.3.3 Phenotyping Screening of Amaranth Germplasm for Salt and Drought Tolerance

Precision phenotyping for drought and salt tolerance in a diverse amaranth germplasm resource is an important step towards harnessing the potential of amaranth as a future climate-smart crop (Rao 2002). The development of rapid and reliable phenotyping technique requires proper experimental design, familiarity with diverse germplasm, a target plant age and environments, and able to screen large number of plant varieties as short time as possible (Johnsin and Asay 1993; Cobb et al. 2013). It is always challenging to develop tolerant cultivars due to the phenotypic variations, environments and developmental stages among other factors that may influenced the growth of the plants. Surrogate traits can be a complementary strategy for selection of tolerant cultivars through correlations between yield and yield-related traits (Hoyos-Villegas et al. 2017).

Selection should target genotypes with relatively high yields under both stress and non-stress conditions. The comparative yield performances between stress and non-stress conditions are most often used to quantify the level of tolerance of a genotype rather than a direct selection criterion (Farshadfar and Sutka 2002). Tolerance indices are either based on stress resistance or susceptibility of genotypes (Fernandez 1992). It provides a measure of stress based on loss of yield under stress conditions in comparison to normal conditions (Mitra 2001). Appropriate selection of tolerance indices will be able to differentiate genotypes into four groups criterion; (i) Group A: genotypes with high yield under both non-stress and stress conditions, (ii) Group B: genotypes with high yield in non-stress condition but low yield in stress condition, (iii) Group C: genotypes with high yield in stress condition but low yield in non-stress condition and Group D: genotypes with low yield in both stress and non-stress conditions (Fernandez 1992). The indices have been used in many crops, for example maize (Mhike et al. 2012), durum wheat (Talebi et al. 2009) and sugar beet (Sadeghian et al. 2000).

For the past several years, Sarker et al. had made tremendous efforts in phenotyping the nutritional and antioxidant content of a large *A. tricolor* germplasm from different eco-geographic regions of Bangladesh (Sarker et al. 2014, 2015, 2016, 2017, 2018; Sarker and Oba 2020). Genotypes that were well-adapted and cultivated by local farmers with high yielding potential, and high nutritional content and antioxidant were selected for detailed screening under drought and salt stress. At high salinity stress, *A. tricolor* remarkably enhances its nutritional quality such as protein, micronutrients, β-carotene, ascorbic acid, total polyphenol content (TPC), total flavonoid content (TFC) and total antioxidant capacity. Some of these components such as phenolic compounds, β-carotene and carotenoids demonstrate an excellent non-enzymatic antioxidant for ROS detoxification resulted from severe drought and salt stress, and this increment are the sign of tolerance in amaranth.

We are also conducting trials for identification of drought tolerant genotypes of *A. tricolor* germplasm from AVRDC and USDA Genebanks (Jamalluddin et al. 2021). Forty-four amaranth genotypes of 120 *A. tricolor* core set were initially selected for their variation in qualitative traits such as leaf and stem colours (Fig. 8.1) and genetic diversity based on DArT-Seq generated SNPs. Several physiological parameters have been evaluated for indirect selection of tolerant amaranth, including re-watering assessment, chlorophyll fluorescence and leaf gas exchange (Fig. 8.2). Preliminary finding of this trial revealed that susceptible/tolerance of vegetable amaranth is not associated with geographical origin, genetic diversity and morphological traits (Fig. 8.3). The study demonstrated that physiological response and yield of amaranth is a complex trait. The growth of amaranth in irrigated and water stress conditions was significantly influenced by environmental conditions. Moderate stress intensity maybe more suitable for the identification of drought tolerant amaranth and their possible surrogate traits. The differences of above-ground biomass partitioning into leaves and stems may be a compromise for drought adaptation in stress recovery, and dark-adapted quantum yield (Fv/Fm) could be a useful parameter for identifying drought tolerance in amaranth. Strong negative correlations between stem biomass and days to recovery provide evidence that one of the possible strategies of amaranth is an increase in stem biomass to compensate plant growth after re-watering. Further studies are required to quantify stem traits and chlorophyll fluorescence of diverse genotypes, and this could be done using a pool of well characterized drought tolerant and a contrasting set of drought susceptible genotypes. This is a valuable preliminary data to initiate marker-assisted selection and trait introgression of amaranth under drought-stressed and non-stressed conditions.

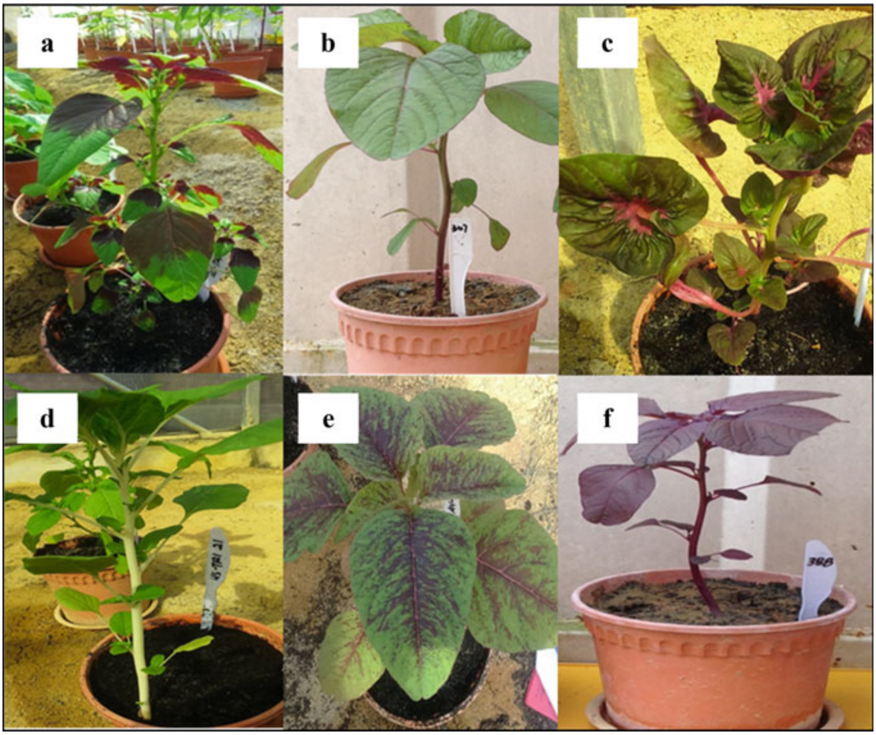

Fig. 8.1 The variability of characters among selected 44 *A. tricolor* accessions: **a** basal area pigmented leaf, **b** vein pigmented leaf with red stem, **c** pink spotted leaf with pink petiole and stem, **d** green leaf with white petiole and stem, **e** green leaf with spotted purple, and **f** perfect red amaranth

8.4 High Quality Reference Sequence: Accelerated Genome Enabled Breeding for Superior Drought and Salt Tolerant Cultivars

8.4.1 Amaranth Reference Genome: Assessing Phylogenetic Tree and Genome Synteny

8.4.1.1 Whole-Genome Sequencing

Sunil et al. (2014) produced a highly fragmented draft genome of *A. hypochondriacus* (Rajgira), a cultivated grain landraces from southern India. The draft genome contained 367,441 scaffolds with an N50 of 35 kb and 40% larger than the predicted genome size of 431.8 Mb (Bennet and Smith 1991) or 500 Mb (Lightfoot et al. 2017). The second amaranth genome assembly *A. hypochondriacus* (Plainsman), a cultivated grain amaranth variety in the United States by Clouse et al. (2016) produced

Fig. 8.2 **a** Fourty-four *A. tricolor* accessions arranged in a split plot design for drought phenotyping assessment, including **b** chlorophyll fluorescence parameter (Fv/Fm), dark-adapted leaf with blackout paper for 20 min at pre-dawn

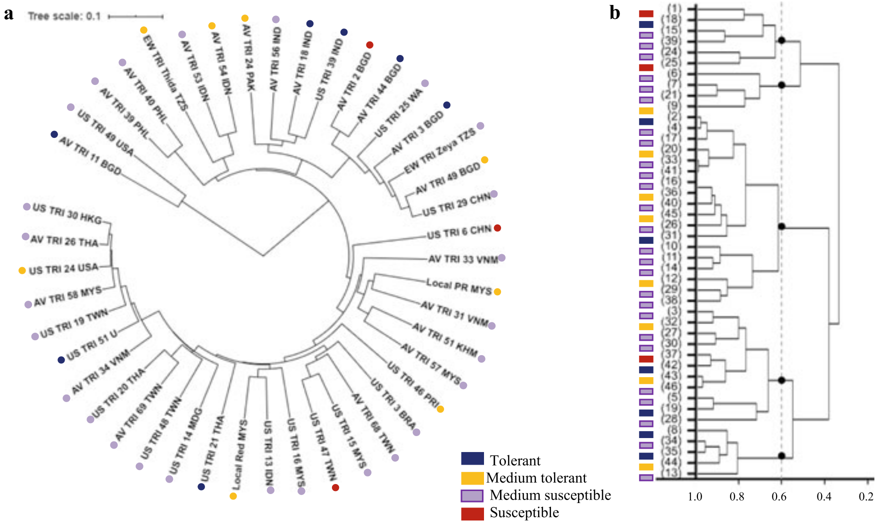

Fig. 8.3 The distribution of susceptible/tolerant amaranth genotypes under drought conditions, **a** phylogenetic tree based on DArT-Seq SNP-based and **b** phylogenetic tree based on qualitative traits (leaf, stem and inflorescence color)

substantially more contiguous but still highly fragmented 3518 scaffolds with an N50 of 371 kb and contained 377 Mb, smaller than the predicted genome size. The genome assembly showed 48% of the genome is comprised of repetitive elements and 1.8% identified as SSR (Mallory et al. 2008). This sequence consists of over 3000 scaffolds are not assembled into the 16 chromosomes of the species.

Later, Lightfoot et al. (2017) produced a very high-quality sequence genome, highly contiguous, 16 chromosome-scale assemblies of *A. hypochondriacus* (Plainsman), with contig and scaffolds N50 of 1.25 and 24.4 Mb, respectively. The 16 chromosomes ranged in size from 17.0 to 38.1 Mb. The total sequence length of the assembly spanned is 403.9 Mb, representing 93.5% of the predicted genome size. This sequence provides an anchor to all the SNP loci and allele sequences discovered. This reference genome facilitates the study of amaranth chromosomal evolution by comparing amaranth genome with beet genome and enable mapping of the betalain locus responsible for stem color, allowing for genotype-to-phenotype relationships. The results from this genome assembly indicated that *Amaranthus* underwent whole genome duplication before speciation, which was then followed by further duplication, chromosome loss and fusion events (Behera and Patnaik 1982; Stetter and Schimd 2017).

More recently, Deb et al. (2020) produced high quality and cost effective de novo assembly of *A. hypochondriacus* (A.hyp_K_white, previously known as Rajgira) using low-coverage PacBio reads, contigs from the previous reported draft genome (Sunil et al. 2014), and raw HiC data and reference genome of *A. hypochondriacus* Plainsman (Lightfoot et al. 2017). The utilization of this assembly is proven with the generation of lysine expressions profile through transcriptome mapping and successfully reclassify amaranth species. This landrace was found to be genetically distal from Plainsman and closely related to landraces originated from India and South Asia, therefore this assembly offers a better reference genome for amaranth crops in Asia. This landrace is currently used in targeting induced local lesions in genomes (TILLING)-based approach to identify novel mutations in targeted loci for a given desirable phenotype.

8.4.1.2 Chloroplast Genome Sequencing

The chloroplast is maternally inherited and non-recombinant, therefore, low-coverage sequencing is sufficient for phylogenetic reconstructions (Zhang et al. 2011; Davis et al. 2014; Chaney et al. 2016). The high copy nature, highly conserved gene order and content across different plant families, and relatively small size make chloroplast sequences an ideal target for high throughput sequencing (Stull et al. 2013). The amaranth chloroplast genome retains the quadripartite structure and highly conserved at the nucleotide level among the grain amaranths (Chaney et al. 2016; Hong et al. 2019). To date, several full-length chloroplast sequences of amaranth have been completed, including grain (*A. hypochondriacus*, *A. cruentus*, *A. caudatus*), vegetable (*A. tricolor*) and weed (*A. palmer*, *A. hybridus*, *A. retroflexus*) (Chaney et al. 2016; Viljoen et al. 2018; Hong et al. 2019; Sharpe et al. 2020; Yan Xu et al. 2020), through different methods such as PacBio technology and Illumina technology. The sequenced of all chloroplast genomes revealed INDELs, polymorphic SSR and informative SNP marker.

Viljoen et al. (2018) demonstrates that whole chloroplast phylogenomic could differentiate closely related amaranth species better than using the three multi-locus barcoding genes (matK, rbcL and ITS), and found nine gene functions encoded for photosynthesis. This sequence was able to place *A. tricolor* and other amaranth species correctly within Caryophyllales family together with beet root and spinach. The sequence identities, gene organization and relative positions of the genes in *A. tricolor* and other amaranth species were consistent with previous studies of the nuclear genome (Stetter et al. 2017), the presence of betalains pigments conferring leaf, stem and flower color (Cuenoud et al. 2002, Venskutonis and Kraujalis 2013) and C_4 photosynthetic pathway. However, poor resolution of amaranth species due to their complex genetic structure and on-going domestications restrict the use of chloroplast data for resolving recent evolution. Because of the highly conservative nature of chloroplast genome, they tend not to integrate with foreign DNA fragments (Cui et al. 2021) hence, recent evolution in amaranth can only be obtained through the nuclear markers. Nevertheless, care should be taken when investigating amaranth nuclear markers due to unknown selection pressures, on-going gene flow and hybridization among amaranth species. High ploidy levels of amaranth species may be characterized by a high level of heterozygosity, and the presence of multiallelic SNPs can influence phylogenetic interpretations.

The development of a reference chloroplast genome in *Amaranthus* is important for broader comparative studies within Caryophyllales family of different type of oxygenic photosynthesis (C_3 and C_4 pathway) and open doors for chloroplast gene transformation, a useful tool for expressing targeted genes in chloroplast with enhanced abiotic stress tolerance. Chloroplast genome information can also be used to resolve hybrid incompatibility as a result of mismatched nuclear-chloroplast genome preventing the gene flow. The chloroplast-nuclear incompatibility can affect photosynthetic capability which drive to speciation, as observed in primroses (*Oenothera* spp) (Zupok et al. 2021). A rich exchange of genetic material from the chloroplast to the nucleus has made up 90% of the protein functioning in the chloroplast are encoded by the nucleus including genes involve in photosynthesis (Sharpe et al. 2020). Currently, most enzymes are involved in C_4 and C_3 cycles are nuclear encoded except the gene for the large subunit of Rubisco, which is in the chloroplast genome, except for the small subunit gene is in the nucleus (Offermann et al. 2011; Wimmer et al. 2017). Therefore, by analyzing the sequence homologies between chloroplast and nuclear genomes in amaranth, basic data related to chloroplast-nuclear communication can be elucidated.

8.4.2 *Genome Wide Association Studies (GWAS), Quantitative Trait Loci (QTLs) Mapping, Genomic Selection (GS)*

Breeding programmes established for amaranth have just begun and the development of diversity panel through NGS may be able to facilitate trait dissection and gene discovery for tolerance in amaranth (Brenner et al. 2010; Alemayehu et al. 2014; Stetter et al. 2016). The genome of amaranth is relatively small (500 Mbp) and diploid, making it easy to study potential genetic improvement and whole genome resequencing of hundreds of individuals (Stetter and Schimdt 2017). This is particularly important for assessing diversity of amaranth germplasm and use this diversity to map QTLs by GWAS (Joshi et al. 2018). The goal of GWAS is to discern genomic regions that could either be markers, genes or QTLs associated with important economic traits for marker-assisted breeding, gene discovery or gene introgression (Edae et al. 2014). The complete assembly of the amaranth genome may accelerate annotation framework and gene discovery, whether through traditional biparental mapping (Lightfoot et al. 2017) or GWAS (Stetter et al. 2020). Both studies identified MYB-like transcription factor genes regulating the betalain red pigment pathway, which gives rise to seed color variations (Gates et al. 2016). These genes were present in the QTLs on chromosome 3 and 9, and once target regions/genes are identified, breeding method will be enhanced, and cloning and marker-assisted selection can be more effectively employed.

However, drought and salinity phenotyping traits are complex and often low heritability, and the marker trait associations can be located on regions that influence the respective traits directly or indirectly (Mwadzengini et al. 2016). SNP loci that associate with similar traits under normal and stress conditions will ideally not be influenced by the environmental variations (Mathews et al. 2008). This SNP with related traits may be due to the correlation among the traits or due to pleiotropic effect of specific genomic regions on more than one trait (Jabbari et al. 2018). Such genomic regions could be useful in marker-assisted selection or gene introgression when breeding for broad adaptation (Mwadzengeni et al. 2016). Precautions should be taken when handling GWAS as it may lead to at least one false negative and false positive. This is because, the difficulty of working with plant genomes is that they are highly repetitive and feature excessive structural variation between members of the same species, mostly attributed to their active transposons (Bennetzen 2000). For example, in the well-studied species *Arabidopsis thaliana*, natural accessions are missing 15% of the reference genome, indicating a similar fraction maybe absent from the reference, but present in other accessions (1001 Genomes Consortium 2016). Moreover, although *A. thaliana* has a small (140 Mb) and not very repetitive genome compared to many other plants, SNPs may be assigned to incorrect positions due to sequence similarity shared between unlinked loci (Long et al. 2013). Therefore, more excessive structural variation is expected in a larger *A. tricolor* genome (782.7 Mbp) with highly repetitive and has undergone ancient and recent rounds of polyploidization (Stetter and Schmid 2017).

GS is another way to identify and develop stress tolerance in amaranth. The GS approach uses a large number of SNPs to predict genomic estimated breeding values (GEBV) without having to test them in the field, and recently has been utilized for stress tolerance in many crop species, including maize (Shikha et al. 2017). It reduces the selection time by half a cycle, in which only a subset of individuals is phenotyped and genotyped to train the genome-wide model. The estimated markers can be used to predict GEBV to other individuals through genome-wide marker profile (Desta and Ortiz 2014). With the assembly of high-quality sequence genome, individual with superior GEBVs selection can be performed. In order to exploit the full potential of GS in amaranth breeding, advanced breeding lines of amaranth is required and the choice of phenotypic traits conferring stress should be accurate and reliable.

8.5 Conclusions

By the virtue of unique nutritional profile, C_4 photosynthetic pathway and genetically diversity, amaranth is a crop well-suited to diversify the agricultural system. Amaranth is a climate-smart crop, has the ability to survive in salt and drought stress and in some extent, stress may enhance the nutritional quality. With the availability of the high-quality reference genome sequence of *A. hypochondriacus*, new targets of selection become more feasible through genome-wide data.

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Chapter 9
Genomic Designing for Abiotic Stresses in Carrot (*Daucus carota* L.)

Raman Selvakumar and Pritam Kalia

Abstract Stress tolerance is the outcome of the plant's reaction to abiotic stresses, which is a dynamic and multi process involving many interdependent systems. While it has been widely explored in model plant species, it has not been systematically investigated in carrot. Only a few studies have been published that show the role of certain proteins in response to stresses. There has been no effort to define the regulatory systems that regulate carrot tolerance to heat, cold, drought, salt, and other abiotic stresses. Nonetheless, the problem seems to be critical, as agriculture deals with global climate change. Furthermore, the global region of carrot production is expanding, and its adaptability to environmental circumstances beyond the temperate climatic zone might give health advantages to malnourished human populations. In this chapter, we review the available information on carrot's response to abiotic stresses, with a focus on molecular or genetic processes influencing stress tolerance.

Keywords Abiotic · Carrot · Environment · Heat · Cold · Drought · Salt

9.1 Introduction

One of the world's top ten vegetable crops is carrot [*D. carota* L. subsp. *sativus* (Hoffm.) Schübl. & G. Martens] ($2n = 2x = 18$). Over the past 50 years, carrot crop yields and production area have increased dramatically, with Asia leading the way (Simon 2019). This increase in production is most likely due to carrots' monetary value for farmers as well as their superior nutritional qualities for consumers. Carrots are a good source of dietary nutrients, with provitamin A carotenoids, which give them their orange color, being the most abundant. Vitamin A deficiency has been a

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serious global health problem for decades, with only relatively insignificant improvements in recent years, but there is still a significant demand for dietary vitamin A in the majority of the world's impoverished communities, including areas of Asia where carrot production has increased (Tanumihardjo 2012).

Carrots were formerly a popular root vegetable in Central Asia, and they are now grown and maintained as an annual crop, with winter root crop production collected before floral initiation. Carrot production was moved to the summertime 600 years ago when it was introduced to Northern Europe, and selection was made for a lack of immediate bolting. Carrot is usually regarded a cool-season biennial crop due to its large production in cooler temperate countries (Banga 1963; Simon 2000); however, considering its origin in Central Asia (Iorizzo et al. 2013), carrot may maintain some genetic variety for adaptability to warmer climatic conditions. Despite the fact that carrot cultivars suited to higher temperatures have been created in southern Asia from the beginnings of carrot as a root crop, the optimum growth temperature for a carrot crop is 15–18 °C, with a maximum recommended temperature of 24 °C. (Maynard and Hochmuth 2007). Carrot cultivar selection for warmer regions has received a lot of attention in South America in recent century, as shown by the creation of the heat-resistant 'Brasilia' cultivar, which helped Brazil significantly increase carrot productivity (Vieira et al. 1983). Abiotic stress tolerance in carrots has received cursory research (Bolton et al. 2020; Grzebelus 2019).

9.2 Abiotic Stresses in Carrot

Abiotic stress is one of the most important challenges that plants face in natural environments, as well as the most yield-limiting problem that agricultural farming faces. The present trend of increasing global temperatures is projected to continue (Pachauri and Reisinger 2007), aggravating the difficulties of heat stress, drought, and salt stress for crop productivity encountered by farmers who must simultaneously raise output to feed a growing worldwide population. Numerous studies have found that poor growing conditions can reduce crop productivity by 50% or more (Boyer 1982; Rockström and Falkenmark 2000), emphasizing the importance of crop improvement to improve abiotic stress tolerance as part of developing strategies to sustain and increase global food supplies. To address this need, researchers are working to improve abiotic stress tolerance across a wide range of staple and feed crops, as well as fruits and vegetables such as carrots and other cool-season vegetables. Plants' reaction to abiotic stresses include a complicated reprogramming of an array of genes, which are triggered by stress perception and result in physiological changes that enable plants to survive the stress period. Cross-talk of several key components is required for stress sensing and plant resistance to unfavorable conditions, including abscisic acid (ABA) (Qin et al. 2011), redox homeostasis (Foyer and Noctor 2005; Gill and Tuteja 2010), soluble sugars (Rosa et al. 2009), membrane localized receptor-like kinases (RLKs) (Osakabe et al. 2013), and calcium (Knight and Knight 2001). The activity of transcription factors is affected by stress perception and signal transduction, which alters the expression levels of genes directly implicated in the physiological response (Chinnusamy et al. 2004). Furthermore, epigenetic regulation of abiotic stress response is possible. Stress may cause histone modifications and DNA (de)methylation, altering chromatin state and driving changes in gene expression (Chinnusamy and Zhu 2009). The establishment of durable epialleles and improved long-term transgenerational adaptability to poor environmental circumstances may result from epigenetic reprogramming that extends beyond the time of stress (Mirouze and Paszkowski 2011). Small RNAs have a function in both epigenetic modification and turn out to be progressively regulation of plant genes in response to stimuli (Khraiwesh et al. 2012; Sunkar et al. 2012).

Studies on molecular processes of response to abiotic stresses are famously difficult due to the existence of the above-mentioned interdependent processes, and they need a large amount of fundamental information on the genome biology of the researched species. Its why, since the dawn of the “post-genome age” in plant research, most of the fundamental processes have been documented for model plant species for which substantial genetic data have been accessible. The availability of genomic information in carrot was constrained until recently, resulting in just a scattering of knowledge on possible genetic factors influencing abiotic stress tolerance. In this chapter, we’ll go over some of the latest studies on the effects of abiotic stressors on carrots, as well as some of the hypothesized genetic routes for resistance.

9.2.1 Heat

Carrots, being a cool-season crop, may be especially susceptible to high temperatures, which is one of the main abiotic factors restricting germination at all stages. Vieira et al. (1983) examined carrot seed germination under heat stress (35 °C) in a variety of different genotypes, finding significant diversity in heat tolerance (Vieira et al. 2005; Pereira et al. 2007; Nascimento et al. 2008). Despite the fact that there has been little research on carrot thermotolerance, a few processes and potential genes have been proposed. The first, alternative oxidase (AOX), is an enzyme that has been shown to alleviate oxidative stress induced by reactive oxygen species (ROS) production. More recently, Bolton et al. (2019) discovered significant diversity in heat tolerance in a collection of 308 carrot germplasm accessions and breeding lines, the majority of which were grown.

As carrot cultivars are developed for cultivation in warmer regions, the influence of high temperatures on early crop growth has been explored. Nascimento et al. (2008) and Bolton et al. (2019) found that, compared to the control temperature of 24 °C, germination of most carrots was reduced by at least 50% up to 35 °C, although several OPs examined found no significant decrease in germination at 35 °C compared to 24 °C. At 37.5 °C, only “Brasilia” seed germinated, but only at a rate of less than 10% of the cultivars tested. Temperature can reach at which the carrot root crop can survive during stand establishment and crop growth after germination have not been recorded.

Heat tolerance may have a significant role in carrot seed production in addition to root crop development. Broussard et al. (2017) showed a decrease in volatile terpenoid production and nectar quality in flowering carrots exposed to "cold," "average," and "warm" greenhouse temperatures, which was hypothesised to reduce bee pollinator attractiveness. Because adequate seed generation is crucial to crop productivity, more research into the subject is needed. Impacts of climate change on the reproductive phase of the carrot life cycle will be critical.

Stress-induced cell conversion in plants is important for adaptability under hostile climates. This carrot alternative oxidase (AOX) mechanism has been well researched. The OX genes may be able to regulate the formation of reactive oxygen species (ROS) in the mitochondria as a response of heat stimuli (Nogales et al. 2016). There are a lot of plant species that only have two copies of the *AOX* gene for each of the two *AOX* families, *AOX1* (two paralogs) and *AOX2* (two paralogs), but the carrot has four genes that correspond to each of the two *AOX* families, with each of the *AOX1* and *AOX2* genes present in double copies. This gives it an advantage for studying the importance of *AOX* in stress response (Costa et al. 2009). It has been observed that carrot *AOX* genes respond greatly to temperature fluctuations. Increasing the temperature of the callus tissue from 21 to 28 °C resulted in a five-fold increase in the expression of *DcAOX1*. Following exposure to chilling, both *DcAOX1* and *DcAOX2a* were both shown to have dramatic expression changes within seconds of exposure (Campos et al. 2016). The current allelic variability in *DCAOX1* was shown to be sensitive to environmental control, which might mean that the allelic variability might influence the host's resistance to abiotic stresses, making the gene a candidate for marker-assisted selection (MAS) in carrot breeding (Nogales et al. 2016). The molecular markers for *DcAOX2a* have also been identified (Cardoso et al. 2009).

Plant reactions to abiotic stresses need the presence of heat shock proteins (HSPs). They serve as molecular chaperones protecting other proteins from fluctuations in temperature. Heat shock protein *DCHSP17.7* is found in carrots and is extensively researched about its protective effects. Prior to its discovery, carrot was thought to serve a crucial function in tolerating high temperatures (42 °C). The modification of the *DcHsp17.7* transcript levels could explain the varied responses to climate variability and change (Malik et al. 1999). In a study done by Park et al. (2013), it was shown that the enzyme was created immediately and sustained itself up to two days after being exposed to heat, but thereafter degrades. During night exposure, the *DcHsp17.7* increased compared to the same stress administered during the day. Interestingly, at 2 °C, *DcHsp17.7* was also shown to accumulate, which is associated with *DcHsp17.7*'s role in regulating cold tolerance (Song and Ahn 2010). Heat shock variables in carrot have been examined more extensively by Huang et al. (2015). Under heat stress, most *DCHF*s are upregulated, but the effect of cold stress is more varied. While being upregulated by cold, the *DCFHsf09* gene appears to be downregulated by heat.

Kumar et al. (2013) employed suppression subtractive hybridization (SSH) to identify and clone genes that are activated by cold stress. Stress resulted in the creation of new proteins that coded for proteins involved in signal transduction, transcription regulation, translation, conformational changes, and osmotic adjustment production.

This study found the two genes that code for carrot laccase, *DcLac1* and *DcLac2*, are regulated in distinct ways depending on the therapy. A kind of copper-containing enzyme, laccases, might be implicated in plants' resistance to environmental stresses. Because of the observed changes in their expression profiles in carrots exposed to different temperatures, salts, and metals, it is possible to conclude that they are essential (Ma et al. 2015).

9.2.2 Cold

Resulting from climate change temperature differences that are not just above but also below recent averages. Carrots are known to be cold resistant, surviving temperatures as low as 8 °C. In addition to leaf injury, cold temperatures cause taproot cracking in carrots. Palta and Simon (2004) observed differences in leaves and roots injuries across parental genotypes and employed screening to decrease taproot cracking rate. The creation and dissemination of two frost-resistant combination genotypes.

The mechanism for freezing tolerance has been explained, and the primary constituent of that ability has been found (Smallwood et al. 1999). It utilizes the polygalacturonase inhibitor protein (*PGIP*) gene family member, the antifreeze protein (AFP). PGIP enzymes, notably AFP, are secreted endocytosis. Although the ability of AFP to form ice-entangled crystals has yet to be confirmed, it's possible that this is due to an unique function that allows it to interact with ice by producing ice-entangled crystals and blocking ice phase transformation in the periplasm (Meyer et al. 1999). Carrot AFP has been proven to enhance cold resistance when introduced to plants through gene editing.

9.2.3 Drought

Agriculture is extremely vulnerable to reduced rainfall and changing rainfall patterns (Fahad et al. 2017). Reduced leaf water potential and decreased cell growth are common drought stress symptoms in plants, which have an unfavorable effect on plant development as well as a variety of physiological and biochemical processes including as photosynthesis, nutrition metabolism, respiration, and chlorophyll production (Hussain et al. 2018). As global warming continues, the impact of drought on carrot production will only grow. A dcTLP gene that encodes a thaumatin-like protein (TLP) was found to be specifically increased during dehydration. ABA, SA, and JA had no effect on it (Jung et al. 2005). TLPs are pathogenesis-related (PR) proteins that are engaged in a plethora of stress responses, including those produced by abiotic stresses. Because *DcTLP* may play a critical role in physiological adaptation of carrot plants to drought, it may be one of the fundamental factors influencing plant's response to drought. During a drought-induced gene discovery in the carrot somatic embryogenesis callus, Jung et al. (2005) identified degraded DNA-binding protein, dilation factor, GTP-binding protein, mitochondrial, and myosin PfM-B-like protein. In addition, *DcHsp17.7*, a heat shock protein, has been shown to be significantly elevated during osmotic stress, hinting at its relevance to defense against abiotic stresses in carrot (Ahn and Song 2012).

Only a few studies on carrot growth in drought have been published. Drought caused production loss and substantial changes in sugar content and other nutritional composition in carrots. Given the persistent lack of rainfall and decreasing availability to sufficient quality irrigation water in recent decades, comprehensive field performance data assessing the impacts of dryness on carrot production would be useful.

9.2.4 Salinity

As a salt-sensitive glycophytic plant, carrot has long been recognized as one of the most salt-sensitive vegetable crops (Bernstein and Ayers 1953; Maas and Hoffman 1977). Carrot production decreases by around 14% for every unit rise in salinity over the 1.0 dSm^{-1} threshold, which is considerably lower than the stated threshold of 4 dSm^{-1} for a saline soil. Increased salt concentrations in the soil also have a negative impact on carrot seed germination and seedling establishment (Schmidhalter and Oertli 1991a, b). Under salinity stress, both total seed germination capacity and rate of germination are substantially reduced, with these effects becoming more pronounced as salt concentration rises (Kahouli et al. 2014). Salinity stress has also been shown to decrease photosynthesis and stomatal conductance rates in carrots (Gibberd et al. 2002). Similar to heat stress tolerance, little research has been done to uncover mechanisms of carrot salt tolerance, but several have been proposed.

Soil salt has a detrimental impact on carrot germination and seedling development in general. (Schmidhalter and Oertli 1991a, b). Carrot plants under salt stress showed reduced rates of photosynthesis and stomatal conductivity (Gibberd et al. 2002). Elevated levels of glycinebetaine (GB), malondialdehyde (MDA), and ascorbic acid, and impaired peroxidase, catalase, and superoxide dismutase activity, all resulted from salt stress (Bano et al. 2014). It is conceivable that abscisic acid mediates salt tolerance by inducing the *DcPSY2* gene (ABA). Carotenoids, which are produced in carrot roots, are precursors of ABA. Salt stress and ABA induce the expression of *DcPSY2* via binding to ABA-responsive elements (*ABRE*s) found in the promoter of *DcPSY2*, according to Simpson et al. (2018). Although, *DcHsp17.7* may possibly be a component of the plant response to high salt, we expect to see this protein in plants exposed to very high salt levels (Song and Ahn 2011).

Because most of the world's carrot production is irrigated, and rising salt levels are becoming more of a concern, assessing genetic diversity in carrot germplasm for salinity tolerance may offer significant insights into the carrot crop's future possibilities for higher salinity tolerance. Previous assessments described limited collections of cultivated carrot, and they discovered carrot to be a salt-sensitive crop during seed germination in the cultivars studied (Bernstein and Ayers 1953; Maas and Hoffmann

1977). A more recent study discovered significant diversity in carrot seed germination under salt stress and identified germplasm accessions that germinated successfully with NaCl concentrations as high as 150 mM (Bolton and Simon 2019). Kahouli et al. (2014) discovered up to 70% yield decrease among 10 Tunisian carrot accessions for both seed germination and crop yield of field-grown carrot irrigated with salty water. Rahim and Mannan (2018, 2019) and Mannan (2020) have observed variance in carrot seed germination and plant development in the field under heat, drought, and salt stress. Furthermore, Ali et al. (2019) discovered differences in drought tolerance across 19 carrot germplasm accessions.

In a collection of different carrot accessions, researchers discovered a broad range of phenotypic differences for salt tolerance during the germination stage. Five farmed carrot accessions (PI 509433, PI 652374, PI 652402, PI 652403, and PI 652405) were found as salt-tolerant, while inbred lines B493B and Nb6526B, as well as four wild accessions (PI 177381, PI 279764, PI 652344, and PI 652380), were identified as extremely salt-sensitive. These accessions may be used to create mapping populations to discover the quantitative trait loci (QTL) related with salt tolerance during the carrot germination stage. The finding of salt-tolerant farmed accessions bodes well for breeders since they may be utilized to create salt-tolerant cultivars. The creation of salt-tolerant carrot cultivars will offer farmers worldwide with more tools for growing on salt-affected soil. This salt tolerance assessment of various carrot germplasm offers important information for future carrot salt tolerance research (Bolton and Simon 2019).

Certain wild *Daucus carota* subspecies have evolved successfully to growing in salty environments. However, it may be difficult to establish salt tolerance into cultivars from wild crop relatives. There have been descriptions of carrot landraces of Iranian provenance that are very resistant to saline stress (Kasiri et al. 2013). The experiment combining in vitro selection for salt tolerance and subsequent examination of regenerants for response to high salinity indicated that Iranian carrot populations had a greater salt tolerance. The Flakkee type western carrot cultivar 'Dolanka' showed a significantly higher survival rate than plants developed from one of the two Iranian populations. Simultaneously, in protoplast cultures, 'Dolanka' showed a significantly wider range of variation in reaction to salt tolerance selection than eastern carrot populations, resulting in the development of more tolerant seedlings. Increased anthocyanin pigmentation of petioles, and also leaf and petiole hairiness, were associated with enhanced salt tolerance, implying possible genetic and epigenetic processes (Kiełkowska et al. 2019). The ability to germinate on a medium supplemented with 150 mM NaCl was used to distinguish F2 populations generated from a cross between such a western-type carrot breeder line and an eastern-type salt-tolerant entry. On the NaCl-treated medium, only about 20% of seeds sprouted, compared to 98% on the control conditions. Additionally, salt-stressed seedlings had a distinguishable morphological characteristics than seedlings grown in a salt-free media, with the former being denser, greenish yellow, and swelled up. Boron (B) is often found in high quantities in saline soils, causing additional abiotic stress to crops. The morpho-physiological responses of carrots to salinity and boron stress were studied by Eraslan et al. (2007). Salt concentration stress on its own enhanced root diameter and reduced root dry weight, but when combined with boron stress, the tendency was partly reversed. When salt and boron stress were combined, the negative physiological changes were more pronounced. Treatment with salicylic acid (SA) reduced boron stress to some extent. In vitro cultures were also used to investigate the effects of elevated boron on carrot cells. Glyoxalase 1 (a detoxification protein), glutathione peroxidase, isocitrate dehydrogenase, and adenosyl homocysteinase were observed as accruing in strained cells, the large number of which are engaged in redox homeostasis, but also, and this is interesting, carrot has major allergen *Dau c*1 and extracellular dermal glycoprotein (EDPG) precursor (Demiray et al. 2011). Previously, the latter was expected to be produced quickly in response to lesions (Satoh et al. 1992).

9.2.5 Heavy Metals

The accumulation of heavy metals in carrot roots has been analyzed since they are detrimental to individuals. Heavy metal content safety guidelines have been developed in most countries, with an emphasis on cadmium and lead. The standards are significantly stricter for carrots used in the preparation of infant food. When cultivated in contaminated soils, heavy metals, particularly cadmium, may be easily retained in carrot roots at amounts much surpassing those criteria. Carrot cultivars absorb significant levels of cadmium and exhibit minimal variation in their susceptibility to cadmium absorption. Thus, carrot production on cadmium-contaminated soils should be avoided (Zheng et al. 2008). Agriculture techniques, such as fertilization, may alter the intake of heavy metals (Smoleń and Sady 2006, 2007). Heavy metals, in addition to being harmful to human health, may put the plant under a high levels of stress. Sanità di Toppi et al. (2012) demonstrated in vitro that continued exposure of carrots to high cadmium concentration levels tends to result in the production of an adaptive mechanism designed to limit heavy metals movement in the rhizome, followed by gradual root degradation. However, no genetic mechanism for enhancing carrot tolerance to heavy metals has been identified. Since carrot heat shock proteins increase in plants lead-exposed and arsenic, they may enhance resistance to heavy metal stress (Park et al. 2013).

9.2.6 Hypoxia

Hypoxia, or a deficiency of oxygen in the root system, is an abiotic stress that may impede plant growth and productivity. According to conventional agricultural methods, it seems to be of minor importance in carrots. Que et al. (2018), on the other hand, used hydroponics to investigate the effect of hypoxia on carrot root development. Carrot roots became more attempts to describe when grown in a low-oxygen environment, according to the researchers. Three alcohol dehydrogenase genes (ADH1–3) were found to be substantially up-regulated in hypoxic roots as contrasted to roots grown in oxygenated conditions.

9.3 Transcription Factors Regulation

Numerous transcription factor (TF) gene families have been found and characterized in carrot. Around 10% (3267 genes) of all annotated carrot genes were found to have regulatory activities. When compared to other plant species, the carrot genome expanded substantially in six transcription factor families (ZF-GFR, JmjC, TCP, GeBP, B3, and response regulators) (Iorizzo et al. 2016). Their methods of action are clearly distinct, and they control a wide range of functions, from embryonic to adaptive. Several TF groups were further investigated in order to identify their potential role in plant abiotic stress tolerance. The APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factor family has 267 members in the carrot genomes. In response to abiotic stressors, eight randomly selected AP2/ERF genes from different subfamilies exhibited significant effect and unique transcriptional regulation (cold, heat, salinity, and drought). Their symptoms differed depending on the kind of stress, as well as the date of accession (Li et al. 2015). It was also discovered that the basic helix-loop-helix (bHLH) transcription factors are involved in plant responses to abiotic stressors. In carrot, 109 genes encoding bHLH proteins were discovered. Eight bHLH genes (identified as subfamily 15) showed varied expression, both accession and tissue-specific, and in reaction to several types of plant responses. They were mostly increased in response to heat, cold, and drought, but their reaction to salt stress differed depending on the species (Chen et al. 2015). The DNA-binding one zinc finger (Dof) family was found to have 46 genes in carrot. Members of this TF family were found to have extremely varied and irregular translation pattern with respect to biogenic stimulus, which may represent their unique reaction to a specific stress (Huang et al. 2016). Another well-known family of transcription factors in plants is WRKY, which controls a range of activities in plants, including plant response to abiotic stressors. In carrot, 95 WRKY genes were found, with 71 of them being expressed, the minority of them in a chronologically coordinated way. Twelve carrot WRKY genes were selected based on their Arabidopsis orthologs' presumed involvement in the response to abiotic stressors. Their expression profiles were examined in cold, temperature, salinity, and drought-stressed crops. The amount of transcription varied depending on the gene and the type of stress, with certain genes showing an 80-fold increase (DcWRKY27/salinity stress) (Li et al. 2016). The examples above show how important it is to look into the molecular pathways that govern carrot responses to abiotic stresses. The overall characteristics of stress-related TF families are just the initial step in explaining key regulatory mechanisms. Data on TF expression is still scarce, necessitating concerted research.

9.4 Exploration of Wild Relatives for Abiotic Stresses

A research to evaluate carrot crop wild relatives (CWRs) was initiated in 2015, based on the wide genetic, morphological, and ecogeographic variability in wild carrot that had been successfully used in carrot development. Bolton and Simon (2019) and Bolton et al. (2019) reported on screening salinity and heat tolerance in carrot CWRs during seed germination; Rahim and Mannan (2018, 2019), and Mannan (2020) reported on aspects of carrot growth as influenced by heat, drought, and salinity tolerance; and Ali et al. (2019) reported on aspects of drought tolerance (2019).

The majority of the research on carrot stress tolerance has focused on evaluating carrot germplasm during seed germination. Abiotic stress tolerance must be successful in the field for the whole growing season in order to be beneficial to farmers. They provide research on the field performance of 66 CWR accessions, including subsp. *carota*, subsp. *capillifolius*, subsp. *gummifer*, and *D. syrticus*, cultivated under abiotic stress conditions in Bangladesh and Pakistan (Simon et al. 2021).

In certain temperate countries, carrot has been extensively cultivated, although attempts have been made to create new cultivars suitable for warmer locations. Carrot culture was successful in Brazil with the use of highly suited native landraces from European origin, which is ideal for production in the subtropical environment. The open-pollinated '*Brasilia*' crop is a large portion of carrot output and its derivatives (Simon et al. 2008). The clarification of the main genetic determinants of adaptation to Abiotic stress and the inclusion of molecular tools in breeding would certainly reduce the time for the development and selection, and support previous efforts, of plant materials with desired qualities for production in areas with malnutrition and vitamin A deficiency. Using molecular approaches (e.g. backcrossing with marker supports) might potentially help to transmit the abiotic stress tolerances in the wild *Daucus carota* gene pool more efficiently.

9.5 Genetic Engineering

Genome engineering improves the use of carrot in transgénesis (Baranski 2008) and CRISPR/Cas9 genome editing (Klimek-Chodacka et al. 2018). The second method has not been used to modify plant responses to abiotic stresses since it was discovered fairly recently. However, genetic transformation has been used to improve carrot sensitivity, and there have been many papers on the expression of exogenous stress-related genes in carrot. Betaine levels were found to be significantly higher in transgenic carrot plants that included a gene coding for BADH (Kumar et al. 2004). The mammalian 6-phosphofructo-2-kinase/Fru 2, 6-P2ase (6-PF-2-K/Fru 2, 6-P2ase) gene was used to transform carrots, which resulted in much higher levels of Fru-2, 6-P2 in the transgenic plants' roots. Drought and cold exposure both required the production of anaerobic glycolysis. In genetically modified carrots, this nicotine gene was also responsible for the increased drought tolerance (Annon et al. 2014).

9.6 Bioactive Compounds

The synthesis of secondarily imported secondary metabolites in the protective response may be increased on the basis of abiotic stresses after harvest. These molecules may be collected and industrialized thereafter, since they demonstrate a spectrum of functions that are advantageous to human health. The quantity of phenolic compounds and carotenoids contained in carrot roots was increased by heat shock and UV-C irradiation (Alegria et al. 2012). UV-B therapy and the consequent *DcPAL1* overexpression, a critical gene for phenylpropanoid biosynthesis, have resulted in substantial phenolic accumulation three days following treatment with shredded carrots (Formica-Oliveira et al. 2017). Expression of *DcPAL1* and the accelerated buildup in stored carrot roots of 3-O-caffeo-caffee-lquinic acid and isocoumarin and ethylene (del Rosario Cuéllar-Villarreal et al. 2016) were elevated as well. Maeda et al. (2005) reported on a proposed method for modulation of the expression of *DcPAL1* after UV exposure. UV therapy has raised *DcMYB1*'s expression by encoding a transcription factor of MYB that increases the *DcPAL*'s expression1. The wounding of *DcPAL1* and its composition was caused in combination with ethylene and methyl jasmonate therapy, depending on the severity of the wound (Heredia and Cisneros-Zevallos 2009). In conjunction with the loss of water, phenylalanine and 3-deoxy-D-arabino-heptulosanate synthases were overexpressed and phenol buildup followed (Becerra-Moreno et al. 2015). ROS probably contribute significantly to the buildup of phenolic compounds in carrots that suffer injury because they upregulate genes that encode essential phenylpropanoid enzymes (Han et al. 2017). Sánchez-Rangel et al. (2014) have created a bioactive chemical extraction process, principally chlorogenic acid and its products, manufactured from carrot roots after the wound.

9.7 Conclusion

The ability of carrots to withstand abiotic stresses is an area of research that's also mostly unexplored. As indicated, there is limited existing understanding that most evidence indicates the potential relevance of certain proteins, which are more completely defined by tiny heat shock protein *Hsp17.7* and antifreeze protein AFP. The regulation of stress reactions is not systematically identified for carrot, with the exception of some very early evidence concerning alterations in expression in response to the abiotic stresses of a few TF families. There is an urgent need for well-designed studies that integrate current information based on research into model plant species with contemporary genomic techniques. Plant materials with contrasting stress responses should be used in conjunction with omic analyses, which include genomics (e.g., genome-wide association studies, or GWAS), transcriptomics (transcriptomic modifications), methylomes, and smaller RNA sequencing, to elucidate the mechanisms governing their tolerance to abiotic stresses, as well as other approaches.

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