Chittaranjan Kole *Editor*

Genomic Designing for Abiotic Stress Resistant Pulse Crops

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Dedicated to

Prof. R. S. Paroda

Former Secretary to the Department of Agricultural Research & Education, Government of India, and Director General, Indian Council of Agricultural Research, and the Founder Chairman, Trust for Advancement of Agricultural Sciences (TAAS)

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

Preface

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

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

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

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

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

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

There are obviously a number of reviews and books on the individual aspects of plant molecular breeding, genetic engineering, and genomics-aided breeding on crops or on agro-economic traits which includes the 100-plus books edited by me. However, there is no comprehensive reviews or books available that has coverage on crop commodity groups including cereals and millets, oilseeds, pulses, fruits and nuts, vegetables and technical or industrial crops, and modern strategies in single volumes with precise focuses on biotic and abiotic stresses. The present volumes will fill this gap with deliberations on about 120 important crops or their groups.

This volume on "*Genomic Designing for Abiotic Stress Resistant Pulse Crops*" includes nine chapters focused on Common Bean, Chickpea, Pea, Cowpea, Lentil, Pigeonpea, Faba Bean, Asiatic Beans and Grass Pea contributed by 80 scientists from 9 countries including Australia, Ethiopia, India, Lebanon, Morocco, Spain, Sudan, UK and USA. I remain immensely thankful for their highly useful contributions.

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

New Delhi, India Chittaranjan Kole

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Abbreviations

100SDW	100-seed weight
^{13}C	Carbon isotope 13
4-Cl-IAA	4-Chloroindole-3-acetic acid
AA	Ascorbic acid
ABA	Abscisic acid
ABC	ATP-binding cassette transporter
AB-QTL	Advanced backcross QTL
ABRII	Agricultural Biotechnology Research Institute of Iran
ABTS	2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate)
ACC	1-Aminocyclopropane-1-carboxylate
ADH	Alcohol-dehydrogenase
AFLP	Amplified Fragment Length polymorphism
ALSV	Apple latent spherical virus
AMF	Arbuscular mycorrhizal fungi
AMOVA	Analysis of marker variance
AOX	Alternate oxidase
AP	Ascorbateperoxidase
ARC	Agricultural Corporation Center
ATP	Adenosine triphosphate
BAC	Bacterial artificial chromosome
BES	BAC end sequence
BIBAC	Binary BAC
BLAST	Basic local alignment search tool
BM	Biomass
BOAA	b-N-oxalyl-amino-L-alanine
BPMV	*Bean pod mottle virus*
BSA	Bulked segregant analysis
BSR-Seq	Bulk segregant analysis RNA-Seq
CAD	Cinnamyl alcohol dehydrogenase
CaMV35S	*Cauliflower mosaic virus* 35S
CaMV35SDE	Double enhanced *Cauliflower mosaic virus* 35S

Cas	CRISPR-associated protein
CAT	Catalase
CBF	C-repeat Binding Factor
CBL	Calcineurin B-like protein
CcHyPRP	*C. cajan* hybrid proline rich protein
CcMT1	*Cajanus cajan* metallothionein 1
cDNA	Complementary DNA
CGKB	Cowpea Genespace/Genomics Knowledge Base
CGMS	Cytoplasmic-genetic male-sterility
CGR	Crop growth rate
CIPK	CBL interacting protein kinase
Clp	Chaperone protein
cM	CentiMorgan
CMS	Cell membrane stability
CNV	Copy number variation/variant
CO_2	Carbon dioxide
COX	Cytochrome oxidase
CRISPR	Clustered regularly interspaced short palindromic repeats
cry1AcF	Crystal protein 1AcF
cry1E-C	Crystal protein 1E-C
CWR	Crop wild relative
DArT	Diversity array technology
DAS	Days after sowing
DEG	Differentially expressed gene
DF	Days to 50 % flowering
DH	Doubled haploid
DHN	Dehydrin
DII	Drought intensity index
DM	Days to maturity
DOGMA	Dual organellar genome annotator
DREB	Dehydration responsive element binding
DSB	Double stranded break
DSI	Drought susceptibility index
Dt1	Growth habit locus
DTI	Drought tolerance index
EBI	European Bioinformatics Institute
EGS	Early generation selection
EMBO	European Molecular Biology Organization
EMS	Ethyl methane sulphonate
EREBP	Ethylene-responsive element binding protein
ESP	Exchangeable sodium percentage
EST	Expressed sequence tag
ETR	Electron transport rate
F_2	Second filial generation
FAC	Florigen activation complex

FACE	Free air CO_2 enrichment
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FD	Frost damage
FIGS	Focused identification of germplasm strategy
FOP	*Fusarium oxysproum* f. sp. *phaseoli*
FRAP	Ferric reducing antioxidant power
FT	*Flowering Locus T*
GA3	Gibberellic acid
GAB	Genomics-assisted breeding
GATT	General Agreement on Tariffs and Trade
GBS	Genotyping-by-sequencing
GCIM	Genome-wide composite interval mapping
GCP	Generation Challenge Program
GEBV	Genomic estimated breeding value
GEI	Genotype-by-environmental interactions
GIS	Geographic information system
GM	Genetically modified
GMO	Genetically modified organism
GMS	Genetic male-sterility
GP	Gene pool
GP1	Primary gene pool
GP2	Secondary gene pool
GP3	Tertiary gene pool
GPX	Glutathione peroxidase
GS	Genomic selection
GSS	Gene space sequences
GST	Glutathione S-transferase
GWA	Genome wide association
GWAS	Genome wide association study/studies
GxE	Genotype x environment interaction
H_2O_2	Hydrogen peroxide
HDR	Homology dependent repair
HI	Harvest index
HSF	Heat shock factor
Hsfs	Heat shock protein transcription factors
Hsp	Heat shock protein
ICARDA	International Center for Agricultural Research in Dry Areas
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
INRAE	Institut National de la Recherche Agronomique
IPR	Intellectual property right
iProClass	Integrated Protein Classification
IR	Inverted repeat
IRAPs	Inter-Retrotransposon Amplified Polymorphism
ISR	Intron spanning region

ISSR	Inter-simple sequence repeat
iTRAQ	Isobaric tag for relative and absolute quantification.
KASP	Kompetitive allele-specific PCR
KEGG	Kyoto Encyclopedia of Genes and Genomes
LAI	Leaf area index
LC-MS/MS	Liquid chromotography-mass spectrometry
LD	Linkage disequilibrium
LEA	Late embryogenesis abundant
LER	Leaf expansion rate
LG	Linkage group
LIS	The Legume Information System
LOD	Logarithm of odds
LRR	Leucine-rich repeat
LWP	Leaf water potential
MAB	Marker assisted breeding
MABC	Marker-assisted backcrossing
MAGIC	Multi-parent advanced generation intercross
MAPK	Mitogen-activated protein kinase
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MDA	Malondialdehyde
MIM	Multiple interval mapping
miRNA	MicroRNA
MLE	*Moringa oleifera* leaf extract
MOOB	Multi-objective optimized genomic breeding
MPPs	Multi-parent populations
MSI	Membrane stability index
MTA	Marker-trait association
NADH	Nicotinamide adenine dinucleotide
NAM	Nested association mapping
NAR	Net assimilation rate
NBPGR	National Bureau of Plant Genetic Resources
NBS-LRR	Nucleotide-binding site leucine rich repeat
NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
NPBTs	New plant breeding techniques
NPs	Nanoparticles
NREF	Non-redundant reference
NUE	Nutrient use efficiency
O_2^-	Superoxide radicals
OA	Osmotic adjustment
ODAP	Oxalyldiaminopropionic acid
ODR	Oxygen diffusion rate
OH	Hydroxyl radical

ORF	Open reading frame
OsRuvB	*Oryza sativa* RuvB
P5CR	L∆1-pyrroline-5-carboxylate reductase
P5CS	Pyrroline-5-carboxylate synthetase
PAGE	Polyacrylamide gel electrophoresis
PAV	Presence/absence variants/variations
PAW	Plant available water
PCD	Pulse Crop Database
PCR	Polymerase chain reaction
PDB	Protein Data Bank
PDR	Pleiotropic drug resistance complexes
PDS	Phytoene desaturase
PEBV	*Pea early browning virus*
PEG	Polyethylene glycol
PEPB	Phosphatidyl ethanolamine-binding protein
PGPR	Plant growth-promoting rhizobacteria
PHS	Pre-harvest sprouting
PHT	Plant height
pI	Isoelectric point
PIR	Protein Information Resource
PKAMs	Pigeonpea kompetitive allele-specific PCR assay markers
POD	Pods per plant
POX	Peroxidase
PP	Pea protein
PPB	Participatory plant breeding
PPN	Pea protein nanoparticles
PPVFR	Protection of Plant Varieties and Farmar's Right
PRL	Primary root length
Pro	Proline
PSD	Protein Sequence Database
Psp68	*Pisum sativum* p68 gene
PSPDB	Plant Stress Protein Database
PUFA	Polyunsaturated fatty acid
PVC	Polyvinyl chloride
PVE	Phenotypic variation explained
PVP	Plant variety protection
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
Rchit	Rice chitinase gene
RDW	Root dry weight
REMAP	Retrotransposon-microsatellite amplified polymorphism
RF	Root forks

Rf	Fertility restoration gene
RFLP	Restriction fragment length polymorphism
RGA	Rapid generation advance
R-gene	Resistance gene
RILs	Recombinant inbred lines
RLD	Root length density
RNA-seq	RNA sequencing
ROS	Reaction oxygen species
RPRV-HN	Hemagglutinin neuraminidase gene of peste des petits ruminants' virus
RPVH	Hemagglutinin gene of render pest virus
RSA	Root surface area
RWC	Relative water content
SA	Salicylic acid
SAMS	S-adenosyl methionine synthetase
SAT	Semi-arid tropics
SB	Speed breeding
SCL	Seed coat luster
SCMR	SPAD chlorophyll meter reading
SCoT	Start codon targeted polymorphism
SD	Short day
SDW	Shoot dry weight
sgRNA	Single guide RNA
SIR	Salinity induction response
siRNA	Small interfering RNA
SMD	Sterility mosaic disease
SMG	Selectable marker gene
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SPAC	Soil–plant–atmosphere continuum
SPD	Seeds per pod
SRA	Sequence read archives
SRAP	Sequence-related amplification polymorphism
SSAP	Sequence-specific amplification polymorphism
SSD	Single seed descent
SSR	Simple sequence repeat
STMS	Sequence tagged microsatellite site
STS	Sequence tagged site
SuSy	Sucrose synthase
SV	Structural variation
SW	Seed weight
SWNTs	Single-walled carbon nanotubes
TAC	Transcript assembly contig
TALEN	Transcription activator like effector nuclease
TbyS	TILLING by sequencing

TDM	Total dry matter
T-DNA	Transfer DNA
TF	Transcription factor
TFC	Total flavonoid content
TFs	Transcription factors
TILLING	Targeting induced local lesions in genomes
TOL	Tolerance index
TPC	Total phenolic content
TRIPS	Trade-related Dimensions of Intellectual Property Rights
TRL	Total root length
TRT	Total root tips
TRV	*Tobacco rattle virus*
TSA	Total root surface area
TTC	Triphenyl tetrazolium chloride
UNEAK	Universal Network Enabled Analysis Kit
USDA	United States Department of Agriculture
V. f.	*Vicia faba*
VIGS	Virus induced gene silencing
WAP	Weeks after planting
WBC	White-brown complexes
WFD	Winter frost damage
WGRS	Whole-genome re-sequencing
WL	Waterlogging
WTO	World Trade Organization
WUE	Water use efficiency
YLD	Yield
YRR	Yield reduction rate
ZFN	Zinc finger nuclease
ZIP	Iron-regulated transporter-like

Chapter 1
Designing Common Bean (*Phaseolus vulgaris* L.) for Abiotic Stress Tolerance

Sandeep Kaur Dhaliwal, Pooja Salaria, Navjot Singh Brar, and Prashant Kaushik

Abstract Common bean (*Phaseolus vulgaris* L.) is an important source of carbohydrates, proteins (~22%), minerals, vitamins (e.g., folate) and fiber. Abiotic and biotic stresses are the constraints to high yield and production of common bean. Varieties resistant to biotic and abiotic stresses are among the major breeding objectives for this crop. Most of the agronomically important traits in common bean are controlled by polygenes and therefore it is imperative to understand the mechanism underlying these characters controlled by quantitative trait loci (QTL). Here, we review and compile the information from the studies related to the identification of QTLs for abiotic stresses in common bean. Successful map-based cloning requires handling of major QTLs that behave more or less like single genes which could be isolated in near-isogenic lines, but it also depends on the unambiguous identification of genotypes by progeny testing. Overall, this information will help the common bean breeders to select a suitable method for detection of the inheritance of quantitative traits controlling abiotic stresses and identify donor genes in germplasm resources to ensure that their utilization through introgression.

Keywords Abiotic stress · Common bean · Molecular mapping · QTLs

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1.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is an important legume grown globally. It is a rich source of carbohydrates, proteins (~22% in seeds), minerals, vitamins (e.g., folate) and fiber (Broughton et al. 2003). The valuable products of this legume are snap beans (harvested before complete seed development), shell beans (harvested at physiological maturity), and dry beans (harvested at complete maturity). It is also referred in English to as French bean, string bean, garden bean, etc. According to the morphology and the coloration of the seed, the common bean varieties are called as pinto, pink, kidney, navy, small red, etc. It is cultivated and consumed in countries on all continents from their homelands in America to the Old World and Australia. Among these countries, eight out of the top ten are considered developing countries (Gepts et al. 2008). Common bean is one of the most widely consumed legumes and improving its vitamin and mineral content can address the malnutrition among the poor population in the developing countries (Blair 2013). Due to the domestication of common bean from wild resources that inhabit a narrow ecological niche, it faces many abiotic stresses within its range of agro-ecological zones (Beebe et al. 2006; Briñez et al. 2017). Thus, abiotic stresses are a major drawback for the production and high yield of common bean. Varieties resistant to abiotic stresses are among the primary breeding objectives for common bean (Ochoa et al. 2006; Cichy et al. 2009).Cultivars possessing resistance against stresses can decrease the yield loss from these stresses, thus, enabling stable production of beans across different environments and diverse soil conditions (Lynch 2011).

The genetically simple forms of resistance can be understood by Mendelian ratios, whereas it is difficult to understand the complex forms, often referred to as quantitative trait loci (QTL) (Lopez et al. 2009). Most of the abiotic stresses are under the control of several genes scattered by several genomic regions harbouring more or less numerous candidate genes. A wide set of molecular markers throughout the genome and dense molecular maps (McClean et al. 2004) are now available in common bean, which provide an opportunity to identify the simple Mendelian factors and complex QTLs governing traits of interest (González et al. 2017). During the past decades, QTLs mapping has been accelerated by new genomic tools such as genomic and transcriptomic sequencing. Sequencing information can be further employed in genome editing and transgenic plants' development that significantly decrease the variety development time in those species in which these technologies are sufficiently fine-tuned. Whereas, given the availability of the common bean's whole genome, this potential expands for this crop (Schmutz et al. 2014; Astudillo-Reyes et al. 2015). The favorable and useful QTLs for abiotic stress tolerance are found scattered in both wild and cultivated populations in different gene pools of the common bean. Several efforts have been made to integrate the different breeding approaches to broaden the genetic base and introduce the QTLs for abiotic stress tolerance (Nietsche et al. 2000; Sartorato et al. 2000; Miklas et al. 2006). This chapter emphasizes on research related to the identification of QTLs for abiotic stress tolerance in common bean.

1.2 Genetic Resources of Abiotic Stress Tolerance

The backbone of every breeding and QTL mapping study in relation to abiotic stress tolerance is the availability of desirable sources of variation, mainly tracked from its wild relatives or landraces adapted to varying stresses over the evolutionary line. The evolutionary history of common bean is shared by two separate gene pools, i.e. Andean gene pool native to South America and the Mesoamerican gene pool originating from Central America and Mexico. Variation in seed size, plant morphology, seed storage protein content, molecular and biochemical markers have categorized two gene pools, which were domesticated in two independent domestication events (Singh 1991; Blair et al. 2006). Thus, a range of abiotic stress resistance genes can be obtained from such extensively diverse germplasm sources. These two gene pools of common bean are further classified into races based on adaptation, morphology and traits of agronomic importance. The Andean gene pool was subdivided into races including Peru, Nueva Granada and Chile, whereas Mesoamerican gene pool includes races Durango, Jalisco, Mesoamerica and Guatemala (Singh et al. 1991; Beebe et al. 2000). The information on crossing compatibility and valuable genes present across the range of bean germplasm in various pools is expected to add valuable QTLs to existing genetic resources.

Common bean trait enhancement is centered on genetic diversity in the reasonably narrow gene pool of improved landraces and cultivars. At the same time, wild relatives and closely associated species have been underutilized. Even though the wild relatives of common bean have been effective in trait improvement against biotic stresses, their use to improve various other quantitative traits has been restricted. The important traits present in wild germplasm include abiotic stress resistance found in wild relatives such as *P. acutifolius*, *P. coccineus* (Beaver et al. 2005) and *P. costaricensis*, whereas *P. parvifolius* and *P. acutifolius* are known to harbour high iron content (Sperotto and Ricachenevsky 2017). Moreover, there is a need to explore more accessions of wild germplasm sources by analyzing earlier determined ecological niches, along with the usage of geographic information system (GIS) data, and expert knowledge.

Nevertheless, gaps in knowledge must be dealt with to facilitate the usefulness in breeding using wild relatives. This presents a challenge; thus, both high and low throughput evaluation techniques are being employed for the characterization of the common bean wild relatives. Appropriate testing environments have to be identified for both field and greenhouse/growth chamber analysis of abiotic stress-related traits and genomic techniques, determining the genome level changes during the breeding program.

1.3 Molecular Mapping of QTLs Underlying Abiotic Stress Tolerance

Common bean shares its evolutionary history with two independent lineages, i.e. Mesoamerican and Andean. Various inter- and intra-gene pool crosses were generated to produce genetic linkage maps for trait mapping in common bean (González et al. 2017). In this direction, a Mesoamerican genotype 'XR-235-1-1' was crossed with Andean genotype 'Calima' to generate a backcross population for linkage mapping. This resulted in a total of 11 linkage groups that contained 224 restriction fragment length polymorphism (RFLP) and some isozyme and protein markers covering 960 cm (Vallejos et al. 1992).

The saturated linkage maps are a key foundation in trait mapping studies. An initial linkage map was developed using a recombinant inbred population derived from BAT93 and Jalo EEP558 (McClean et al. 2004). Linkage maps in corporate genetic information thanks to common markers from other linkage maps, which increases the probability of mapping more loci and fine-tuning the linkage between mapped loci. Furthermore, the same map was enriched with more markers such as expressed sequence tag (EST), simple sequence repeat (SSR),and amplified fragment length polymorphism (AFLP), and aided in the development of new cultivars of common bean by introducing or pyramiding identified genes in the cultivars using marker-assisted selection (MAS) (Hanai et al. 2010). Whereas, saturated linkage maps were developed by Córdoba et al. (2010) carrying out the characterization of SSRs in 89,017 bacterial artificial chromosome end sequences (BES) from the G19833 common bean library. They included a total of 280 SSRs in the linkage map, together with 92 previously mapped BES- and 114 non-BES-derived markers, integrating a total of 8,232 bacterial artificial chromosome clones in 162 contigs from the physical map. In another study, 3,123 ESTs developed from root and leaf cDNA libraries were screened for micro satellites. The study yield 184 new SSR markers and 120 of these new microsatellite markers were evaluated for their capacity to distinguish bean diversity in a germplasm panel of 18 genotypes (Blair et al. 2011).

Single nucleotide polymorphism (SNP) based markers were first developed in the crop using a method in which Roche 454-FLX system (454) was coupled with Illumina Genome Analyzer (GA) for sequencing and high-throughput SNP identification. A Golden Gate assay was developed for genotyping by integrating approximately 800 working SNP markers among the identified SNPs (Hyten et al. 2010). After this study, 84 genic and 10 non-genic regions comprising SNPs were validated using Kompetitive Allele-Specific PCR (KASP) technology across a panel of 70 diverse genotypes previously used in crossing for developing mapping populations and tested for SSR marker as well (Cortés et al. 2011). Tentative orthologous genes (TOG) from legume species were used to develop an Illumina Golden Gate Assay featuring 768 SNP genic loci for its use in fingerprinting. The amplicons of the commonly used genotypes 'BAT93' and 'Jalo EEP558' were used in the study for developing markers (Blair et al. 2013). The Illumina Golden Gate Assay designed in this study was used recently by Blair et al. (2018) to locate the SNPs' genetic and

physical positions, and a new map was compared with the whole genome sequence of common bean.

The recombination rate was estimated using a recombinant inbred line (RIL) population derived from BAT93 x Jalo EEP558, and linkage disequilibrium over the whole genome was calculated based on the SNP marker allele diversity Mesoamerican Andean publically-accessible international diversity panels. An SNP assay named 'SNP chip-BARC Bean6K_3' was developed for SNP genotyping by anchoring 6,000 SNPs obtained from sequencing 17 distinct varieties of common bean with the help of Illumina GAIIx platform (Song et al. 2015). Such bead chips for high throughput analysis were valuable and cost-effective, which can also be tested for cross-species genotyping assays. The development of molecular markers accelerates the process of QTL mapping studies (Fig. 1.1). Generally, biparental homozygous mapping populations are employed for associating markers with traits in most of the studies. There is a need of integrating association mapping, multi parent mapping approach and advanced backcross QTL mapping (in the case of the wild donor) for more reliable and transferable linked markers.

Environment plays a crucial role in developing plants, as all the edaphic and climatic factors run parallel during the growth to bring the crop to maturity. During the course of time, deficiency or excess or any environmental factor causes severe yield

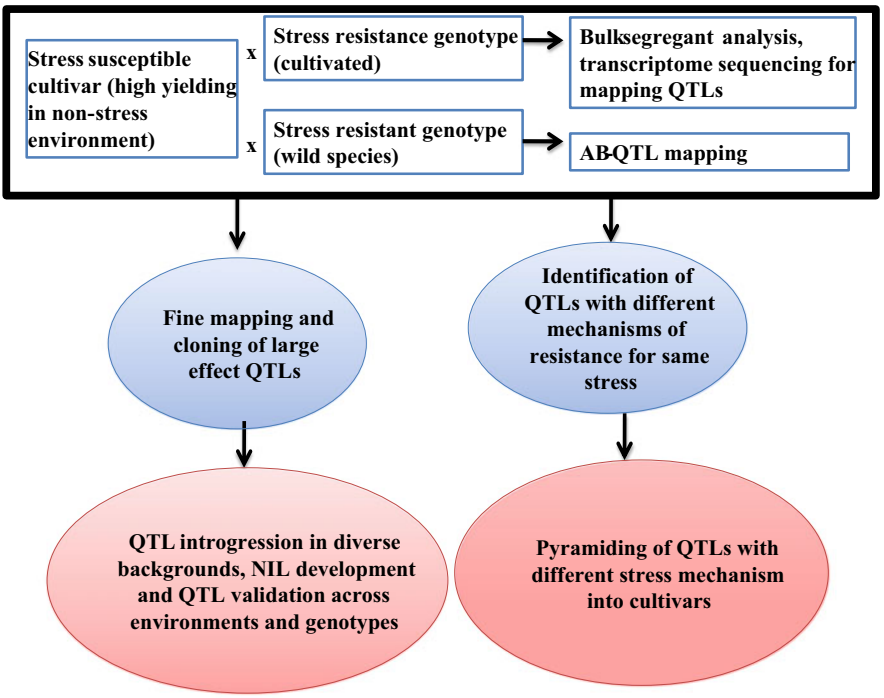

Fig. 1.1 QTL mapping and intro gression strategy for stress resistance

constraints. Drought stress is a significant limitation in yield potential of common bean, especially in subtropical and tropical regions (Rao 2014). Southern Africa and Central America face severe and periodic drought stress challenges in 60% of their growing areas (Ambachew et al. 2015; Darkwa et al. 2016). The significant traits controlling resistance to drought include root depth and size, phenology, carbohydrate mobilization and storage, root hydraulic conductivity, and water absorption (Beebe et al. 2013).Food mobilization traits from primary source to sinks gain breeders' focus during drought stress (Rao et al. 2017). The QTLs for resistance to drought have been mapped across *P. vulgaris* L. chromosomes (Table 1.1; Fig. 1.2).

Heavy metal toxicity such as aluminum (Al) and manganese (Mn) result in elevated levels of acidity in the soil, which is problematic in bean productivity (as in many other crops), especially in Africa and Latin America (Rao et al. 2016). Al toxicity resistance was identified using root traits such as primary root elongation rate, length of roots, and higher diameter of roots in the Andean gene pool (Blair et al. 2009a, b) and QTLs for Al toxicity tolerance were identified by López-Marín and Rao (2009). Soils with low phosphorus (P) are unfit for breaking the yield ceiling of common bean in tropical regions (Beebe 2012). Traits fighting against low soil P include more considerable root length, P uptake efficiency and root area (Ochoa et al. 2006; Rao et al. 2016) (Table 1.1).

Common bean grows well under an optimum range of temperature (14–30 ºC) and day temperature more than 30º or night temperature above 20 degrees is detrimental to bean productivity (De Ron et al. 2016).Blossom drop is a significant problem due to pollen sterility triggered by high temperature and resulting in fewer seeds. Transpiration cooling is achieved by diffusion of carbon dioxide through the stomatal opening at elevated air temperatures (Porch and Hall 2013). Bean genotypes capable of opening stomata at high temperature are valuable sources of resistance to heat stress (Prasad et al. 2017). Therefore, more significant thermal cooling under stress conditions could be an objective for mapping QTLs for heat stress response (McClean et al. 2011; Deva et al. 2020).A total of 50% mortality rate and limited growth after survival were reported at a temperature below freezing temperature (−3.25 ºC) (Meyer and Badaruddin 2001). The sources of resistance to low temperature can serve as a valuable material for QTL mapping in common bean (Rodino et al. 2007; Souter et al. 2017).

1.4 Genomic and Transcriptomic Resources

The advancement in molecular marker technology is directly related to the genomic and transcriptomics resources of a crop. The availability of the genome sequence of common bean has broadened molecular research horizons and ultimately speeded up the cultivar development process (Schmutz et al. 2014). A total 473 Mb-genome was assembled out of total 587 Mb, and 160 genotypes belonging to two different gene pools were resequenced, which included wild species and landraces. The insights of domestication related genes and the abundance of sequence information were

Table 1.1 QTL mapping studies for abiotic stresses in common bean

Molecular marker/QTL	Source	Trait/objective	References
5 RAPD markers	RILs [Sierra × AC1028(S/A), Sierra × Lef-2RB (S/L)]	Drought tolerance	Schneider et al. (1997)
36 QTL for various traits, QTLs on b01 near *fin* locus	RILs (A55 × G122)	Phenological, architectural and yield traits under abiotic stress	Córdoba et al. (2010)
9 QTLs on 6 linkage groups	RILS (DOR364 × BAT477)	Photosynthate acquisition, accumulation, and remobilization under drought	Asfaw et al. (2012)
15 QTLs on 5 linkage groups	RILs (DOR364 × BAT477)	Drought tolerance	Blair et al. (2012)
QTLs, i.e. *SY1.1, SY2.1, PW1.2 BR, NDVI 1.1 BR*	RILs (Buster × Rosa)	Drought tolerance	Trapp et al. (2015)
QTLs *SW*, QTL SY3.3SC on *Pv03*, 14 other QTLs	RILs (SEA5 and CAL96)	Drought tolerance	Mukeshimana et al. (2014)
22 QTLs linked to drought-tolerant traits	F_8 (SEA 5 × AND 277)	Drought tolerance	Briñez et al. (2017)
QTLS *Df1.1, Df 1.2, Dp1.1, Sp2.1, Wp1.1, Wp5.1, Syp1.1, Syp1.2, Sp2*	RILS (Tiber × Starozagorskičern)	Drought tolerance	Sedlar et al. (2020)
2 major QTLs on B2 and B9, 17 minor QTLs	RILs (G2333 × G19839)	Adventitious root traits under low P	Ochoa et al. (2006)
26 QTLs	RILs (G19833 × DOR 364)	Root architecture traits under low P	Beebe et al. (2006)
QTLs linked to *fin* gene	RILs (G19833 and AND696)	Root architecture traits and low P tolerance	Cichy et al. (2009)
24 QTLs	RILs (DOR364 × G19833)	Root morphology traits in Al toxicity	López-Marín and Rao (2009)

obtained from the study for future breeding work. The genome and the transcription atlas of coding and non-coding genes of the Mesoamerican BAT93 genotype of common bean was further published (Vlasova et al. 2016). The genome and transcriptome data generated for a Mesoamerican genotype represented a counterpart to the genomic resources already available for the Andean gene pool. Transcriptomic sequences add additional details of the expressed genome for developing genic

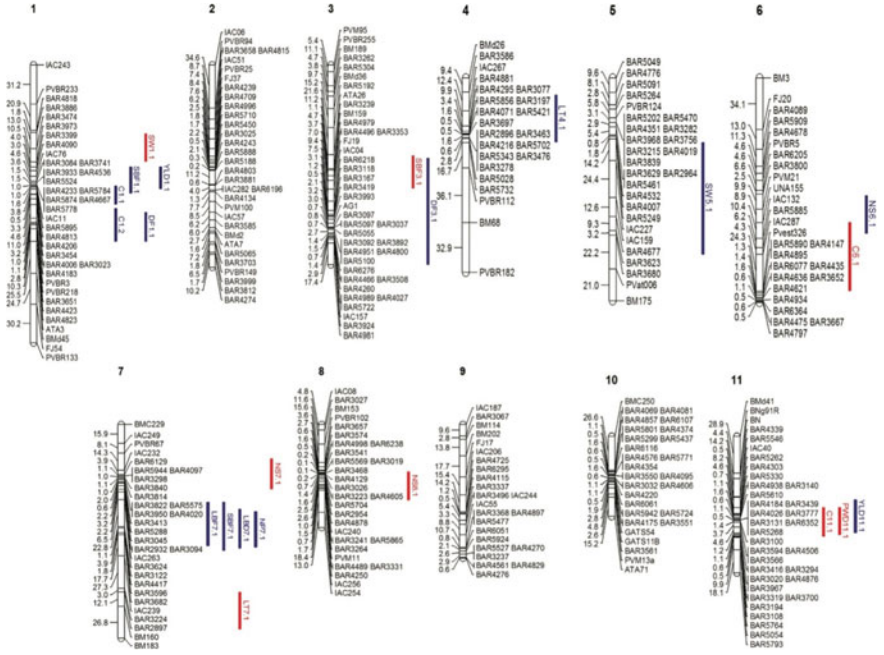

Fig. 1.2 Common bean linkage map from Briñez et al. (2017). This map was produced using 107 recombinant inbred line (RIL) populations obtained from a cross of SEA 5 × AND 277. The QTLs for drought (blue) and irrigated treatments (red) are marked across the chromosomes. Chromosomes were allocated based on the genomic sequence of *P. Vulgaris* L. Available at http://www.phytozome.net/

markers and depict various development pathways in the different stages of plant growth and tissues. Transcriptomes have been sequenced for many abiotic stresses in common bean (Table 1.2).

Zinc (Zn) is an essential micronutrient for higher plants; yet, at higher concentrations it is toxic. Transcriptome sequencing of differential lines (Voyager and Albion) for Zn content was performed using Illumina Genome AnalyzerII. The two cultivars had the same levels of Zinc in their pods and leaves but 52% more Zn was present in seeds of 'Voyager' cultivar in comparison to 'Albion'. RNA sequencing of developing pods revealed that three gene families were involved in Zn transport, i.e., zinc-regulated transporter, iron-regulated transporter-like (*ZIP*), the zinc-induced facilitator (*ZIF*) and heavy metal associated (*HMA*) among a total of 381 differentially expressed genes (DEGs) along with potent SNPs from 11 genes for marker-assisted selection (MAS) (Astudillo-Reyes et al. 2015). Transcriptome sequencing was also performed for revealing genes for resistance to abiotic stresses in common bean, such as salt tolerance, drought resistance and phosphorus responsive genes (Hiz et al. 2014; Pereira et al. 2020). Candidate genes for regulation of energy metabolism, trans-membrane activity and secondary metabolites were identified as

Table 1.2 Transcriptome sequencing and resulting candidate genes for important traits in common bean

Traits	Candidate genes/markers	Breeding strategy	Sequencing platform	References
Zinc content	Three genes involved in Zn transport-*ZIP, ZIF* and *HMA*, 12,118 SNPs	Bio-fortification of common bean for curbing malnutrition	Illumina Genome AnalyzerII	Astudillo-Reyes et al. (2015)
Salt tolerance genes	Candidate genes for regulation of energy metabolism, trans-membrane activity and secondary metabolites identified	Validation and pyramiding of candidate genes in common bean	Illumina HiSeq 200	Hiz et al. (2014)
Drought resistance genes	SSRs and SNPs identified along with differentially expressed genes for drought stress	Useful for breeding Common bean for drought affected areas	Illumina platforms (GAII and HiSeq 2000)	Pereira et al. (2020)
Phosphorus responsive genes	*WRKY*, *ERF*, and *MYB* families, phosphatase related genes	Breeding for phosphorus responsive genes improves the quality of crop and also resistance against biotic and abiotic stresses in Common bean	Illumina Hiseq 2500	Silva et al. (2019)
Spider mite resistance	Flavonoid biosynthesis, pathogenesis-related (PR) proteins and heat shock proteins	Breeding spider mite resistant cultivars	Illumina HiSeq 2500	Hoseinzadeh et al. (2020)

salt-tolerantrelated genes in the crop using Illumina HiSeq 200 of "Ispir", a salt-tolerant cultivar under two variant environments. Among 2,678 transcription factors (TF) identified in the study, 441 were involved in the salt tolerance mechanism (Hiz et al. 2014). The drought resistance mechanism was elucidated by transcriptome sequencing of roots and leaves of two Mesoamerican cultivars (i.e.,Pérola and BAT 477) with contrasting phenotypes for drought tolerance (Pereira et al. 2020). Prominent families of genes involved in drought resistance mechanism were oxidative stress, kinase activity and response to the stimulus. Oxidation–reduction genes were triggered early in the roots of drought-tolerant genotype, indicating a tolerance mechanism by decreasing the damage from reactive oxygen species (ROS). RNA sequencing of IAC Imperador (phosphorus responsive) and DOR 364 (phosphorus unresponsive) in various environments differing for phosphorus concentration was carried out, and *ERF*, *WRKY* and *MYB* gene families were found to be involved in phosphorus restriction along with phosphatase related genes such as acid phosphatase, pyrophosphatase, phosphate transporters and purple acid phosphatase (Silva et al. 2019).

1.5 Prospects and Conclusions

Wild species and crop landraces are goldmines for significant trait improvement in crops. As plant breeding is a number game, one needs to attempt more and more crosses every year with diverse germplasm including wild species and generate mapping populations for obtaining genetic gains and releasing new cultivars. Thus, the populations must be evaluated precisely with advanced phenomic tools, and genomic tools can be employed for dissecting the traits and their functional behaviour. Genomic and transcriptomic studies are growing in common bean, but there is still a need to focus on material used for sequencing and the right stage of plant for transcriptome analysis. The precision in experimental material and methods employed in transcriptomic analysis is the sole criterion for getting accurate and reliable information. Genomic databases for individual crops are being developed, and the purpose of a database is fully released only if there is a balance between outflow and inflow information. The databases are growing as virtual diversity in crop plants and must be used frequently in molecular breeding traits. The amalgam of conventional and genomic techniques generates numerous valuable QTLs. A number of QTL mapping studies for abiotic stresses has been conducted, mostly retaining QTLs specific to a population or environment. To accelerate the desirable genotype development in ever-changing climatic conditions, one needs more stable QTLs. The QTLs express less epistatic background genotype for their efficient marker-assisted backcross breeding programs under varying environmental conditions. The concept of mega-QTLs is a crucial strategy for overcoming the problem and utilizing QTLs among distinct backgrounds. The mapping studies are not entirely accomplished until the product

is not used in developing sustainable genotype. There is an urgent need for comprehensive, collaborative testing of genotypes and QTLs among institutions and countries for validation and deployment in cultivars which is the ultimate goal of plant breeding. The information regarding the genetic basis of inheritance determined by previous studies is useful for the improvement of the common bean is essential. The powerful progression opens brand new research perspectives about the dynamics of combining different traits in one breeding program. This info will help the common bean breeders choose a suitable technique for the inheritance analysis of quantitative characteristics and determine the novel genes in germplasm assets. Overall, the review is an update of common bean genomics and genetics. The vast availability of crop diversity and its utilization to map traits of interest using conventional and genomic breeding has been compiled in the present review. The information is expected to attract advancements in the current scenario of common bean breeding and broaden horizons for future research.

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Chapter 2
A Scintillating Journey of Genomics in Simplifying Complex Traits and Development of Abiotic Stress Resilient Chickpeas

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Abstract Chickpea (*Cicer arietinum* L.) is an important cool season food legume cultivated in more than 55 countries across the globe. In the context of climate change, productivity of chickpea is hampered by higher incidence of abiotic and biotic stresses. Among abiotic stresses, drought, heat, cold and salinity are the most important yield limiting factors. Advanced genomics technologies have great potential to accelerate mapping, gene discovery, marker development and genomics-assisted breeding. Integration of precise phenotypic data along with sequence information

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will help in developing cultivars tolerant to various abiotic stresses. In this chapter, we discuss the impact of various abiotic stresses on chickpea production and provide an update on potential strategies to develop stress-tolerant chickpea cultivars. In addition, we also summarize the systematic efforts of simplifying the complex traits in chickpea as well as development of improved varieties with tolerance to abiotic stresses during last decade. In addition, we also highlight the emerging stresses and future strategies to combat the abiotic stresses.

Keywords Chickpea · Genomics · Abiotic stresses · "*QTL-hotspot*" · Molecular breeding · Transgenic technology

2.1 Introduction

Emerging climate change and the ever-increasing population poses huge challenges to global food and nutritional security. The productivity of agricultural systems are dependent on ability of plants for resisting or tolerating environmental stress (Ferguson 2019). The effects of climate change are already being experienced worldwide, with continuing increases in levels of greenhouse gases and associated rises in temperatures, very likely to reach at least 1.5 °C and possibly 2 °C or more above preindustrial levels by 2050 (Ripple et al. 2019). Globally, high temperature spikes, especially during the most critical reproductive stage of plant growth, are expected to exceed the range encountered during crop domestication (Coyne et al. 2020). With accelerating climate change, increased abiotic stresses are expected to affect global productivity of all major crops and thereby challenge agriculture and food security (Ripple et al. 2019). Furthermore, prevalence of extreme weather conditions is projected to influence pest and pathogen dynamics and compromise defense responses of plants (Atlin et al. 2017). These stresses pose huge challenges for breeders to maintain and improve yield in varying environments (Varshney et al. 2019b). Substantial impacts from environmental change on global legume yields and nutritional quality have been reported (Scheelbeek et al. 2018).

Chickpea (*Cicer arietinum* L.) is an important food legume crop that ranks second in area and production among the pulses worldwide. It is a diploid and predominantly self-pollinated crop cultivated in more than 55 countries in the world. It belongs to the clade Hologalegina of Leguminosae. Among 44 species in genus *Cicer*, *C. arietinum* L. is the only cultivated species. Among 43 wild species, eight species are annual (*C. reticulatum, C. echinospermum, C. pinnatifidum, C. judaicum, C. bijugum, C. cuneatum, C. chorassanicum* and *C. yamashitae*) and 34 are perennial (Sharma et al. 2013). With South East Turkey and Ethiopia as primary and secondary centers of origin, chickpea also believed to possess four centres of diversity (Mediterranean, Central Asia, the Near East and India; Vavilov 1951). Desi and kabuli are major chickpea types, that differ in size, color and surface of seeds, flower color and morphology. Besides being nutritionally rich its nitrogen fixing ability benefits sustainable crop production. Globally during 2018, chickpea was grown in

more than 57 countries and had an area of about 17.81 million ha, production of 17.19 million tons and productivity of 965 kg per ha (FAOSTAT 2018). India is the major chickpea producing country, where chickpea production increased from 3.86 to 11.23 mt between 2000–01 and 2017–18 (Dixit et al. 2019). The other major chickpea producing countries are Australia, Pakistan, Turkey, Russia, Myanmar, Iran, Mexico, Canada and USA.

In past chickpea breeding efforts contributed substantially to improving yield potential, resistance/tolerance to abiotic and biotic stresses, adaptation, plant type and grain characteristics. As per vision document of Indian Institute of Pulses Research, to attain self-sufficiency by 2050, ~39 million tons of pulses needs to be produced in India with an average productivity of 15–17 q/ha (Dixit et al. 2019). Although the production potential of chickpea is about 5 ton per ha, the global productivity is less than 1 ton per ha. This is mainly because chickpea production is hampered by both biotic and abiotic constraints depending on the ecological region. Among abiotic stresses, drought, heat, cold and salinity stresses are the most important yield limiting factors (Boyer 1982; Gunes et al. 2008) that affect chickpea at various stages of plant growth (Fig. 2.1). The complex nature of abiotic stresses coupled with large genotype × environment interaction, physiological and biochemical changes have been hindering the understanding of manipulating these traits for crop improvement.

Advanced genomics next-generation sequencing (NGS) technologies have great potential to accelerate mapping, gene discovery, marker development and genomics-assisted breeding. Integration of precise phenotypic data along with sequence information will help in developing cultivars tolerant to various abiotic stresses. Recent progress in genomics technologies in chickpea has made available greater options to the breeders' toolbox (Roorkiwal et al. 2020). In this chapter, we summarize the systematic efforts of simplifying the complex traits in chickpea as well as development of improved varieties with tolerance to abiotic stresses during last decade. In addition, we also highlight the emerging stresses like dry root rot, root lesion nematode and future strategies to combat the abiotic stresses.

2.2 Abiotic Stresses Affecting Chickpea

2.2.1 Drought Stress

Drought stress is one of the most important abiotic stresses that can reduce yield by about 40–50% as chickpea is largely grown on residual soil moisture by resource-poor farmers in the semi-arid regions. Further, climate changes in recent years are further increasing the frequency of incidence of drought. In the case of drought stress occurring at the vegetative stage, there can be stunted plant growth and limited biomass production. Earlier efforts were made to gain insights into the effect of drought stress on early maturity, root traits, carbon isotope discrimination, shoot biomass (Kashiwagi et al. 2005; Krishnamurthy et al. 2010; Upadhyaya et al. 2011; Ramamoorthy

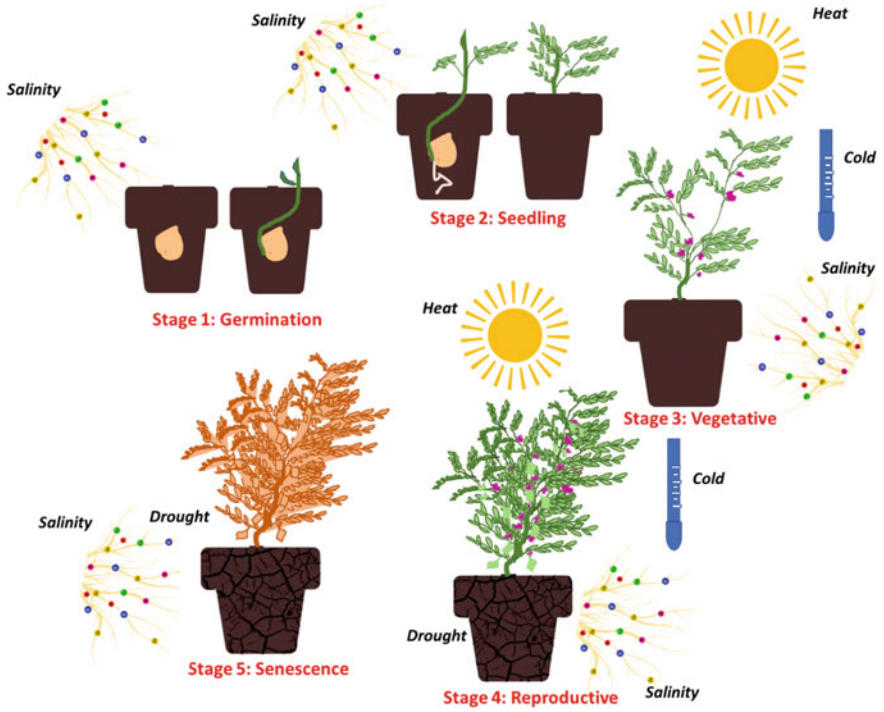

Fig. 2.1 Abiotic stress affecting various stages of chickpea life cycle: Germination and seedling stages are affected by salinity stress. Vegetative stage is affected by heat, cold and salinity stresses. Reproductive stage is sensitive to drought, heat, cold and salinity stresses. Senescence stage is affected by both drought and salinity stresses

et al. 2016), and morphological (Sabaghpour et al. 2006), physiological (Turner et al. 2007) and biochemical traits (Gunes et al. 2006; Mafakheri 2011). Drought stress during the reproductive growth of the plant is termed as a terminal drought. Terminal drought occurs very frequently in the semi-arid tropics (South Asia, north-east Australia) and Mediterranean-type climates such as southern Australia (Li et al. 2018). This situation is more alarming and important for researchers and farmers. There can be drastic reduction in the yield due to malfunctioning of pollen and ovules (Sita et al. 2017). Podding is also affected by reduced pod filling and early abscission of pods, thus reducing the number of seeds per plant. The ability of chickpea to biologically fix nitrogen can also be restrained due to water scarcity (Pang et al. 2017), further impeding crop productivity.

Genetic variation in chickpea for drought tolerance has been studied using various morphological, physiological and grain yield parameters under varied moisture regimes (Jha et al. 2014). To aid selection, stress tolerance indices like drought susceptibility index and drought tolerance index have also been calculated (Krishnamurthy et al. 2010). This kind of selection is done using wild germplasm, landraces and mini-core collections. The role of wild *Cicer* spp. *C anatolicum*, *C microphyllum*

and, *C. songaricum*, in drought tolerance is worth mentioning. The amount of natural variation for drought tolerance traits was studied in a set of 37 landraces, belonging to 14 provinces in the West Asia and North Africa (WANA) region, to develop pre-breeding and breeding strategies for chickpea. Relative water content (RWC) and membrane stability index (MSI) were highly variable in this set of germplasm and the landraces IG5856 (Jordan) and IG5904 (Iraq) were highly tolerant to drought (Tapan et al. 2015). Evaluating training population lines, Sachdeva et al. (2017) inferred that apart from RWC and MSI, Drought susceptibility index (DSI) can be used as an important section index for identification of chickpea genotypes for drought tolerance. Sachdeva et al. (2017) also reported ICC 4958, Pusa 1103 and Pusa 72 to be most drought tolerant. In a separate study (Tapan et al. 2015) identified Pusa 362 to be tolerant and proposed this chickpea genotype to be used for breeding for drought tolerance. Genetic variability has been observed in the chickpea mini-core collection for the before mentioned traits (Chen et al. 2017). Depending on these parameters, genotypes including ICC 4985 and ICC 8261 are identified as donors for improving drought tolerance in chickpea. ICRISAT identified ICC 4985 to be good in root architecture and have a short duration life cycle (Krishnamurthy et al. 2011). Thus, this genotype is used as a reference genome in drought stress chickpea breeding.

2.2.1.1 Physiology of Drought Stress in Chickpea

Understanding the physiology of tolerance to any abiotic stress is very important because these physiological traits have the potential for increasing the genetic gain contributing to plant acclimatization. However, the ability of such traits to increase grain yield depends on the heritability and ease of selection of the target trait (Monneveux et al. 2012). The classical understanding of the mechanisms of drought tolerance by the plant, which include drought escape, tolerance, and avoidance, was given by Levitt (1980). The main physiological traits which help in the abovementioned mechanisms can be categorized into constitutive traits and acquired traits (Sreeman et al. 2018). Water use efficiency (WUE) is defined as the amount of carbon assimilated/biomass accumulated or grain yield per unit amount of water utilized (Hatfield and Dold 2019). The genotypes with lower g_s are reported to be more drought and heat tolerant (Vadez et al. 2012b). Certain biochemical attributes contribute to maintaining osmoregulation in chickpea cells which in turn increases the WUE. Such biochemicals include proline, glutathione, trehalose and antioxidants. The level of proline in chickpea is an indicator of drought tolerance in chickpea. Kaur et al. (2017) studied the differential expression of genes related to proline synthesis in both susceptible and tolerant genotypes of chickpea under water stress conditions. It was recorded that the desi genotype Bakhar-2011 accumulated more proline than Bitall-2016.

2.2.1.2 Mapping Genes/QTLs for Drought Tolerance

Identification and characterization of candidate genes for drought tolerance have been supported by recent advancements in chickpea genomics and genetic engineering (Fig. 2.2).

Linkage Mapping

A major "*QTL-hotspot*" region on chickpea linkage group 4 controlling 12 traits (100-seed weight (100SDW), root length density (RLD), days to 50% flowering (DF), days to maturity (DM), biomass (BM), plant height (PHT), pods per plant (POD), harvest index (HI), ratio between root dry weight (RDW) and total plant dry weight (RTR), shoot dry weight (SDW), seeds per pod (SPD) and yield (YLD) was mapped in two populations, ICC 4958 × ICC 1882 and ICC 283 × ICC 8261 (Varshney et al. 2014) (Table 2.1). This region spanned 29 cM on the genetic map and 7 Mb on the physical map. A high-density map developed using the single nucleotide polymorphism (SNP) data derived from the genotyping by sequencing (GBS) method refined this "*QTL-hotspot*" region to 14 cM on the genetic map and ~3 Mb on the physical map (Jaganathan et al. 2015). Further, this region was fine mapped into two sub-regions "*QTL-hotspot a* and *QTL-hotspot b*" that spanned less than 400 kb (Kale et al. 2015). Root traits and 100SDW are the two important

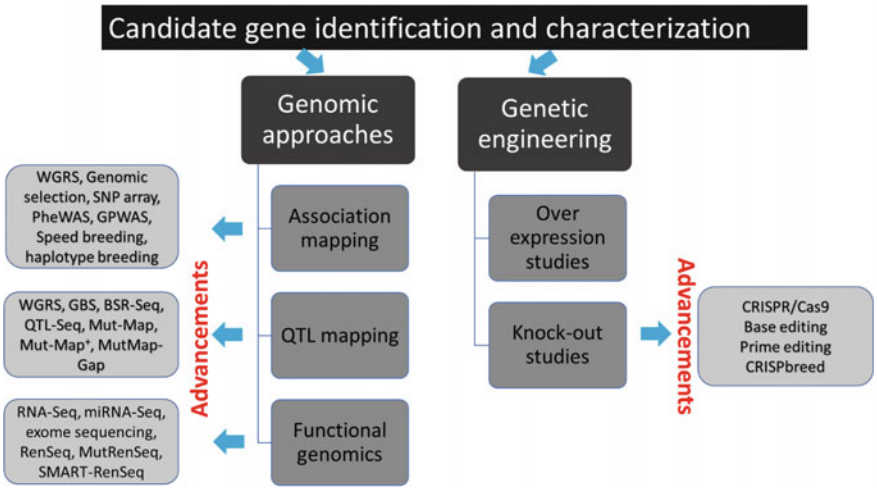

Fig. 2.2 Major advancements made in genomic and genetic engineering approaches for chickpea abiotic stress management. PheWAS: phenome-wide association study; GPWAS: genome-phenome wide association study; WGRS: whole genome re-sequencing; GBS: genotyping by sequencing; BSR-Seq: bulked segregant analysis RNA-seq; QTL-seq: quantitative trait locus-seq; RNA-seq: RNA sequencing; miRNA-seq: microrna sequencing; RenSeq: resistance gene enrichment sequencing; SMART-RenSeq: single-molecule real time RenSeq

Table 2.1 List of studies reported for chickpea trait improvement using advanced genomic tools

Trait	Genotyping platform/approach	Population/ Germplasm	References
Drought	RNA-Seq	ICC 4958	Kudapa et al. (2018)
	WGRS	ICC 4958 × ICC 1882	Sivasakthi et al. (2018)
	Axiom® *Cicer* SNP Array	ICC 4958 × ICC 1882,	Roorkiwal et al. (2018)
		ICC 283 × ICC 8261	
		Bivanij and Hashem	Mahdavi Mashaki et al. (2018)
	WGRS	132 genotypes	Li et al. (2018)
	WGRS	ICC 4958 × ICC 1882	Singh et al. (2016)
	RNA-Seq	ICC 4958 × ICC 17163	Srivastava et al. (2016)
	GBS	ICC 4958 × ICC 1882	Jaganathan et al. (2015)
	WGRS	ICC 4958 × ICC 1883	Kale et al. (2015)
	SSR	ILC588 × ILC3279	Hamwieh et al. (2013)
	SSR	ILC588 × ILC3279	Rehman et al. (2011)
Heat	SSR	71 genotypes	Jha et al. (2018)
	GBS	ICC 4567 × ICC 15614	Paul et al. (2018)
	RNA-Seq	ICCV 92944, ICC 1356, ICC 15614, ICC 5912, ICC 4567, ICC 10685	Kudapa et al. (2017)
Drought and heat	WGRS	Chickpea reference set	Varshney et al. (2019b)
Cold	GBS	ICC 4958 × PI 489777	Mugabe et al. (2019)
	SSRs	ICC4958 × PI 489777	Samineni et al. (2016)
Salinity	Axiom® *Cicer* SNP Array	ICCV 10 × DCP 92-3	Soren et al. (2020)
	SSR	ICCV 2 × JG 62	Vadez et al. (2012a)
Drought and salinity	RNA-Seq	ICC 4958, ICC 1882, JG62, ICCV 2	Garg et al. (2016)
Drought, heat and salinity	WGRS	35 genotypes; DCP 92-3 × ICCV 92944	Thudi et al. (2017), Jha et al. (2021)
Flowering time	SSR	ICCV 96029 × CDC Frontier	Mallikarjuna et al. (2017)
		ICC 5810 × CDC Frontier	

(continued)

Table 2.1 (continued)

Trait	Genotyping platform/approach	Population/ Germplasm	References
		BGD 132 × CDC Frontier	
		ICC 16641 × CDC Frontier	
	GBS	92 genotypes	Upadhyaya et al. (2015)
Seed traits	GBS	SBD 377 × BGD 112	Verma et al. (2015)
Yield	GBS	57 genotypes	Pavan et al. (2017)
	WGRS	ICC 7184 × ICC 15061	Das et al. (2015)
	GBS	93 genotypes	Bajaj et al. (2015)
	GBS	92 genotypes	Kujur et al. (2015a)

traits contributing to yield reduction under terminal drought stress. Two studies have employed QTL-seq to identify the genomic region controlling 100SWD in chickpea. Das et al. (2015) mapped a genomic region for 100SWD on chromosome 1 using an intraspecific chickpea mapping population with 221 lines (desi cv. ICC 7184 × desi cv. ICC 15061). This study also employed QTL mapping and identified the co-localization of the QTL region for 100SWD along with the region identified through QTL-seq. QTL-seq refined the 1.37 Mb region identified through QTL mapping to a 35 kb region possessing six candidate genes on chromosome 1. Similarly, Singh et al. (2016) have employed the QTL-seq approach to refine the already mapped region for the 100SDW and RTR. This study mapped 100SDW and RTR to ~1 Mb region within the "*QTL-hotspot*" region on CaLG04. Several other studies also have explained the importance of root traits in improving yield under drought stress (Chen et al. 2017; Ramamoorthy et al. 2017).

Genome-Wide Association Studies

Re-sequencing of 35 chickpea genotypes, representing parental lines of 16 mapping populations segregating for abiotic (drought, heat, salinity), biotic stresses (Fusarium wilt, Ascochyta blight, Botrytis grey mould, pod borer) and nutritionally important (protein content) traits, was done using the whole-genome re-sequencing approach (Thudi et al. 2016b). A total of 2,058,566 unique SNPs and 292,588 Indels were detected. This study identified genome-wide SNPs, Indels, copy number variations (CNVs), presence/absence variations (PAVs), and miscellaneous variations identified in different mapping populations. These markers can be used for SNP array development and to locate genes/genomic segments responsible for economically important traits in chickpea. Furthermore, the resequencing of 129 chickpea varieties released from 1948 to 2012 revealed CNVs and PAVs contributing to phenotypic variations

and also provided insights into the history of chickpea breeding (Thudi et al. 2016a). Kujur et al. (2015a) reported 44,844 high-quality SNPs by sequencing of 93 diverse cultivated desi, kabuli, and wild chickpea accessions using GBS. This study revealed complex admixed domestication patterns, extensive linkage disequilibrium (LD) estimates and extended LD decay. Further, using the SNP data genome-wide association study (GWAS) was carried out on 211 association panel genotypes, including these sequenced lines (Kujur et al. 2015b). Varshney et al. (2019b) reported the whole-genome resequencing (WGRS) of 429 chickpea accessions, which resulted in the identification of 122 candidate regions with 204 genes. Further, 262 markers and several candidate genes for 13 traits that can be utilized in trait improvement programs. Recently, Li et al. (2018) used GWAS to identify significant association of SNPs from auxin-related genes, including auxin efflux carrier protein (PIN3), p-glycoprotein, and nodulin MtN21/EamA-like transporter, with yield and yield-related traits under drought-prone environments. This study also found that application of SNPs significantly associated with the drought tolerance related traits in agenomic selection (GS) model has increased prediction accuracies of three yield and yield-related traits by more than two-fold. These results have greater implications for application of GS for improving chickpea yield under drought stress conditions. Abscisic acid (ABA) and stress ripening (ASR) genes and their role in insulating chickpea against drought tolerance was reported for the first time by Sachdeva et al. (2020). The characterization and molecular analysis of an ASR gene indicated its conservation in chickpea. This gene was linked to chickpea putative abscisic acid/water deficit stress (ABA/WDS) induced protein mRNA, thereby showing its involvement in imparting drought tolerance in chickpea. The string analysis also identified a hypothetical ASR protein, NP_001351739.1, as a transcription factor.

2.2.2 Heat Stress

Although chickpea is grown as a cool season crop, it encounters high temperature stress in warmer regions and in late sown conditions. High temperature can have a highly destructive effect on growth, biomass (BM), pod set, seed weight (SW), days of maturity (DM) and grain yield (Upadhyaya et al. 2011). The reproductive growth stage is a particularly vulnerable stage for heat stress in chickpea, eventually leading to a loss of yield. High temperatures above 32 °C lead to abortion of floral buds, flowers and pods (Kaushal et al. 2013; Devasirvatham et al. 2015). High temperature stress also results in loss of pollen viability and pollen fertility, which affects pod set and yield (Wang et al. 2006; Kumar et al. 2013; Kaushal et al. 2016). Although heat stress at the vegetative stage is not of major concern, it can affect seed germination. There can be no germination at >45 °C or seedling death if germinated (Kaushal et al. 2013). An extensive study by Devasirvatham et al. (2015) on the effects of heat stress at the reproductive stage indicated reduction in flower number, increased flower abortion, decreased anther locule number, pollen sterility with poor pollen germination, reduced fertilization and stigma receptivity, ovary abnormalities,

reduced remobilization of photosynthates to seeds, reduced seed number, SW and seed yield.

2.2.2.1 Physiology of Heat Stress in Chickpea

Photosynthesis and respiration are vital processes for plant growth. Heat stress is detrimental to both these physiological processes in chickpea. Chlorophyll content, photosynthetic rate, and membrane stability of leaf tissue in chickpea can be used as indicators to know whether the plant is experiencing heat stress (Hasanuzzaman et al. 2013). Membrane stability and photosystem II function in chickpea are more sensitive at a higher temperature of 50 °C for 48 h (Srinivasan et al. 1996). In terms of respiration, due to reduced water content in leaves, stomatal conductance and hydraulic conductance in roots decline (Kaushal et al. 2013). Impairment in floral development of chickpea at a high temperature of $\geq$32 °C is due to partial inhibition of sucrose synthesis enzymes. Chickpeas have indigenous mechanisms for thermotolerance, involving certain antioxidants, acids, chaperons, transcription factors (TFs), etc. The reactive oxygen species (ROSs) produced during heat stress are damaging to the cells. Several antioxidants in chickpea help to sustain this damage during stress and help maintain normal growth. These antioxidants include superoxide dismutase, catalase, ascorbate peroxidase, and glutathione (Kumar et al. 2011). The same authors also found that exogenous application of proline to chickpea exposed to 45 °C increased the levels of before mentioned metabolites. Similar effects were found for salicylic acid (Chakraborty and Tongden 2005) and ABA application (Kumar et al. 2013). Heat shock proteins in plants act as molecular chaperons to protect cellular components during heat stress. Chidambaranathan et al. (2018) identified 22 TFs regulating heat shock proteins. These TFs are called Hsfs (heat shock protein transcription factors) and are classified into three groups. The same researchers performed qPCR and determined that TFs CarHsfA2, A6, and B2 were upregulated at both the early and late stages of growth. CarHsfA2, A6a, A6c, and B2a were identified as early-stage regulators. Apart from these, there are several other proteins and TFs responsible for heat tolerance. However, deeper studies on their mode of action, the differential expression pattern in susceptible and tolerant genotype is required. This information can serve as good knowledge for breeders.

2.2.2.2 Mapping Genes/QTLs for Heat Tolerance

Recently, Paul et al. (2018) mapped QTLs for heat component traits on chickpea chromosomes CaLG05 and CaLG06. This study employed GBS-based SNP markers and developed a high-density linkage map using 292 $F_{8\text{-}9}$ recombinant inbred lines (RILs) developed from the cross ICC 4567 $\times$ ICC 15614 (Table 2.1). QTL region on CaLG05 revealed high PVE (>50%) and identified 25 putative candidate genes for heat-stress were identified in the two major genomic regions. Earlier, Thudi et al. (2014b) reported 312 marker-trait associations for drought and heat response traits

using 1,872 markers comprised of 1,072 diversity array technology markers (DArTs), 651 SNPs, 113 gene-based SNPs, and 36 simple sequence repeats (SSRs). Phenotypic data were generated for 300 accessions, including 211 mini-core collection accessions, for drought tolerance related root traits, heat tolerance, yield and yield component traits from 1–7 seasons and 1–3 locations in India and Africa. This study reported 18 SNPs from five genes (ERECTA, 11 SNPs; ASR, 4 SNPs; DREB, 1 SNP; CAP2 promoter, one SNP and AMDH, one SNP) that were significantly associated with different traits. Association analysis of 71 chickpea genotypes using 81 SSR markers identified genomic regions controlling MSI and leaf chlorophyll content (Jha et al. 2018).

Heat affects the vegetative stage, and especially flowering, and thus the effect on flowering is directly proportional to yield loss in chickpea. Therefore, the development of early flowering is a mechanism to escape major heat stress and results in yield improvement. QTL-Seq approach was employed to identify QTL for flowering time in two mapping populations (ICC 4958 × ICC 8261 and ICC 4958 × ICC 17160). This study narrowed down two major QTL regions into a refined shorter region on chromosome 4 (757.7 kb and 1.39 Mb). Notably, this study reported novel SNPs in two genes (efl1-early flowering 1 and GI-GOGANTEA) regulating flowering time in chickpea (Srivastava et al. 2017). A total of 874 novel differentially expressive genes including a set of 56 APETALA2/ethylene responsive factor (AP2/ERF) and heat shock protein, Indels and SNPs were identified by employing RNA-seq analysis on contrasting heat tolerant and sensitive chickpea genotypes (Kudapa et al. 2017).

2.2.2.3 Breeding for Heat Tolerance in Chickpea

The damage caused by high-temperature stress mainly depends on the plant's defense response and the growth stage (Farooq et al. 2017). The adaptive strategies used by chickpea to avoid, escape and tolerate heat stress (Rani et al. 2019) are not productive in terms of yield. For example, the accelerated phenology under high temperature stress can be considered an escape mechanism, but early maturity is closely related to lower seed yield (Jumrani et al. 2017). Therefore, crop improvement by breeding techniques has been done and is pursued to date. Screening of tolerant genotypes from the pool of germplasm is very important. Breeders have developed several techniques to have a quantitative approach for screening and selecting the best genotypes. Krishnamurthy et al. (2011) developed a simple, cost effective screening technique for heat tolerance by delayed sowing in the field, which enable the plants to be exposed to high temperatures during the reproductive growth stage and accordingly the number of filled pods per plant served as a selection criteria for reproductive stage heat tolerance. Devasirvatham et al. (2013) identified ICC 1561, ICC 1205, and ICCV 92944 as heat-tolerant genotypes, based on pollen selection and pollen viability tests. The heat tolerant chickpea breeding line ICCV 92944 has been released for cultivation in India (as JG 14) Kenya (Chania Desi 2) and Myanmar (as Yezin 6). Based on grain yield among 35 early maturing lines of chickpea, ICC 14346, was selected as a thermotolerant genotype (Upadhyaya et al. 2011). Recently, several selection indices for

heat tolerance viz., heat stress tolerance indices: mean productivity, geometric mean productivity, yield index, tolerance index (TOL), superiority measure, and stress susceptibility index are being used to select the heat tolerant chickpea genotypes. By using these indices, Jha et al. (2018) selected the genotypes RVG 203, RSG 888, GNG 469, IPC 06–11, and JAKI 9218 as moderate to highly tolerant to heat stress.

2.2.3 Cold Stress

Low temperature stress is a major production constraint in chickpea growing regions like northern India, southern parts of Australia and Canada (Kumar et al. 2011). Any temperature below 15 °C for chickpea is considered as chilling stress for growth and development. Chickpea is a cold season crop that can be exposed to low temperatures ranging from 3–8 °C at the time of germination, which is unfavorable for seed germination and seedling establishment. Temperatures can limit chickpea growth and vigor at almost all the growth stages but are most damaging during the reproductive stage (Fig. 2.2). Chilling stress during this phase leads to infertility of the ovule, pollen abortion, poor seed set, pod fill, and finally affects yield. Exposure to low temperatures causes about half of the total productivity in chickpea (Rani et al. 2019). There is scope to select and improve chickpea lines for cold tolerance from existing germplasm during both vegetative and reproductive growth. In a cold-sensitive genotype of chickpea, flower abortion is disastrous. Kiran et al. (2019) documented the effects of cold stress in male and female gametophytes of chickpea and observed that male gametophytic development is more affected than mega sporogenesis. Anther dehiscence, pollen sterility and pollen tube failure were the common features at various stages of floral development. In the case of female gametophyte, poor ovule viability and stigma receptivity were observed. Cold stress in flowers is correlated to increases in ABA content, which affects sugar translocation in flowers and eventually leads to flower abortion (Thakur et al. 2010). These features can be used as indices for developing cold-tolerant lines in chickpea. Berger et al. (2004) have given cold-induced factors that can be used for screening chickpea lines against cold stress: poor plant growth, delayed flowering, flower abortion, delayed pod filling, pod abortion, and poor seed filling.

2.2.3.1 Physiological Traits and Cold Stress

Lower temperatures are hostile to the plant and results in the loss of function of physiological processes. Loss of leaf water content, root hydraulic conductivity, loss of chlorophyll, and damage to membrane potential, adversely affect germination and flowering in chickpea. However cold-tolerant genotypes are found to perform better. Membrane integrity is maintained intact in the case of tolerant genotypes at cold conditions, by increasing the concentration of unsaturated fatty acids content in the plasma membrane (Shahandashti et al. 2013). Also, Karami-Moalem et al.

(2018) described the enzyme alternate oxidase (AOX) to help the plant to establish a separate pathway in mitochondria for ATP production under cold stress. Several ROSs molecules are considered as biochemical markers to screen chickpea lines against cold temperature exposure. Tolerant genotypes show a positive correlation with upregulated expression of ROSs such as catalase, superoxide dismutase, and ascorbate peroxidase. Cold stress for a prolonged period is lethal for chickpea. The physiological means to make chickpea genotypes cold tolerant would be to increase the expression and accumulation of biomolecules that are positively correlated to cold tolerance. Such parameters would be more fatty acids in the membranes, higher levels of antioxidative enzymes, etc.

There must bea merge of information between physiological and genomic studies for a better understanding of mechanisms of susceptibility and tolerance to abiotic stresses. Concerning cold tolerance, there have been few noteworthy revelations. A mitogen associated protein kinase gene (MAPK), *Jk649809*, was found to be responsible for cold acclimatization and cell communication at chilling temperature (Dinari et al. 2013). Sharma and Nayyar (2014) focused on differentially expressed genes during an thesis in chickpea and recorded that genes belonging to signal transduction, carbohydrate, and lipid metabolism were upregulated intolerant lines, suggesting that these pathways were responsible to maintain pollen viability.

2.2.3.2 Mapping Genes/QTLs for Cold Tolerance

Very few studies have reported QTL mapping and association analysis for cold tolerance in chickpea. A major QTL for the vernalization response trait under low-temperature stress was mapped on CaLG03 using an interspecific mapping population (ICC 4958 × PI489777) (Samineni et al. 2016). Sharma and Nayyar (2014) studied the cold tolerance mechanism in chickpea using cold-tolerant chickpea genotype ICC16349. Anther genes controlling cold stress tolerance were identified by expression analysis of stressed and control anther samples of ICC16349. In a recent study, Mugabe et al. (2019) used a mapping population consisting of 129 RILs derived from an interspecific cross between ICC 4958 and PI 489777 to identify QTLs linked to cold tolerance using GBS. A high-density linkage map with 727 SNP markers was constructed and the QTL analysis revealed candidate genomic regions on CaLG1, CaLG3, and CaLG8 (Mugabe et al. 2019). These SNPs associated with QTLs for cold tolerance will assist in molecular breeding. The QTL on CaLG3 was reported earlier by Samineni et al. (2016) using the common parent PI 489777, a cold tolerant and vernalization responsive line.

2.2.3.3 Genetic Variability and Breeding Efforts for Cold Tolerance in Chickpea

The reaction of chickpea on exposure to cold temperature is similar for desi and kabuli lines. However cold conditions vary based on the location and growth stage

of the plant. Though we term it as abiotic cold stress, the temperature in Asia differs from Australia and Mediterranean regions. Thus, the breeding objectives for cold tolerance are highly location-specific. Wild genotypes of *Cicer* spp. have proven to be a credible source of cold-tolerant genes in chickpea (Berger et al. 2012). Efforts have been made to select the best wild *Cicer* relative, both at freezing and chilling temperatures. Previous studies have reported cold tolerant lines within *C. arietinum* (Singh et al. 1990; Wery 1990; Singh and Saxena 1993; Singh et al. 1995). Srinivasan et al. (1998) identified the cold tolerant lines ICCV 88501 and ICCV 88503. Similarly, the lines FLIP95-255C, FLIP93-260C and Sel95TH1716 (Kanouni et al. 2009), and Sel96TH11404, Sel96TH11439, Sel96TH11488, Sel98TH11518, x03TH21, and FLIP93-261C (Saeed et al. 2010) were identified as cold tolerant. The primary gene pool of *Cicer* spp. can be readily crossed with present cultigens. In Australia and the Indian subcontinent, chilling temperature coincides with the reproductive stage of chickpea.

2.2.4 Salinity Stress

Salinity is one of the major abiotic stresses worldwide that limits chickpea crop productivity and which needs serious attention to maintain agricultural production. Nearly 80 Mha of the worlds' arable land is prone to this stress (Flowers et al. 2010). Most crops are greatly affected by salinity, particularly during the vegetative stage (Fig. 2.1). Salinity slows plant growth, leading to reproductive stress and yield loss. Growth is reduced by accumulation of salts in the shoot, resulting in overall reduction in new leaf formation and salt accumulation in older leaves causes premature senescence (Roy et al. 2014). Chickpea is highly sensitive to salinity and an estimated global annual chickpea yield loss of between 8 and 10% is attributed to salinity stress (Flowers et al. 2010). Soil salinity affects anthocyanin pigmentation in leaves of both desi and kabuli chickpea (Millan et al. 2006). In addition, salinity also inhibits plant growth, photosynthesis, metabolism (Parida and Das 2005), flower and pod development (Vadez et al. 2007, 2012a), nodule formation and N_2 fixation (Flowers et al. 2010) in chickpea.

2.2.4.1 Physiology of Salinity Stress in Chickpea

Salinity primarily inhibits growth and development of plants through both shoot ion independent and shoot ion dependent stresses. Immediately upon exposure to salinity, hydraulic resistance is imposed by NaCl in the plant xylem (Munns and Passioura 1984) and the reduction in external osmotic potential (osmotic stress) results in shoot ion independent stress, which interferes with water uptake and leads to a reduction in plant growth rate (Atieno et al. 2017) and ultimately a reduction in shoot biomass. Salt tolerant chickpea genotypes maintain high shoot biomass under salinity stress (Turner et al. 2013; Vadez et al. 2012b). Shoot ion-dependent

stress manifests a few days following exposure to salt, once ions accumulate in the shoot (Munns and Passioura 1984). Mechanisms of salinity tolerance include osmotic tolerance, ion exclusion and tissue tolerance (Roy et al. 2014). Physiological changes during salinity stress in chickpea include changes in photosynthesis, leaf necrosis and increased senescence. During germination, salinity affects both rate and extent of germination, during vegetative growth root development is affected, as compared to shoot development (Flowers et al. 2010) and reproductive process (Samineni et al. 2011). Salinity is also reported to delay flowering time and affect pod filling. Studying variation for salinity tolerance Neeraj et al. (2017) reported a strong relationship between the stem Na:K ratio and yield per plant under salinity. It was inferred that these genotypes could exclude the sodium going to the stem and thus showed better tolerance to salinity. The Na:K ratio in the genotypes CSG8962, ICCV 00104, ICCV 06101 and JG62 showed the minimum damage and better salt tolerance.

2.2.4.2 Mapping Genes/QTLs for Salinity Tolerance

Very few studies have reported QTL/genetic resources for salinity tolerance in chickpea. Identifying sources of salinity tolerance followed by genomic and molecular approaches will result in developing salinity tolerant chickpea varieties. Many studies have attempted to understand the molecular and physiological mechanisms of salinity tolerance in chickpea. For instance, Vadez et al. (2012b) studied the effect of salinity on various stages of chickpea using two sets of five genotypes comprising salt-tolerant and sensitive genotypes. This study reports the key traits including the numbers of flowers and tertiary branches and adaptive traits, like a high number of seeds under salt stress, to be focused to improve salinity tolerance in chickpea. Two key genomic regions on CaLG05 and CaLG07 harboring QTLs for six and five different salinity tolerance associated traits, respectively, were mapped using the RIL population of ICCV 2 × JG 11 (Pushpavalli et al. 2015). Soren et al. (2020) identified candidate genes for salt tolerance using a Axiom® *CicerSNP* array in chickpea in a mapping population of DCP 92–3 × ICCV 10. They constructed a linkage map spanning a length of 1106.3 cM and identified 28 QTLs explaining up to 28.4% PVE in the population. One major QTL cluster each were harboured on CaLG03 and CaLG06. Calcium-dependent protein kinases, histidine kinases, cation proton antiporter, and WRKY and MYB transcription factors were reported to be associated with these QTL clusters. Physiological aspects of salinity tolerance have been reported. However, many of these physiological aspects need to be explored for identifying promising genomic loci for salinity tolerance in chickpea (Atieno et al. 2017; Singh et al. 2018b).

2.3 Advancements in Genomics to Combat Abiotic Stress in Chickpea

Modern genomics technologies have enabled genome assemblies for any crop. Such improvements along with the germplasm collections, prediction of functional genes and gene editing would speed-up breeding to develop climate-smart crops (Varshney et al. 2020). Advanced sequencing technologies allow researchers to perform genome sequencing more accurately and faster, at a cheaper cost. Though modern sequencing technologies provide high throughput reads for assembly, the short reads length and sequence errors are a major concern, especially for repeat sequences (Ruperao et al. 2014). Therefore, assembly validation is a must for any draft genome. The first draft genome of chickpea was released in 2013 on the kabuli type chickpea variety CDC Frontier (Varshney et al. 2013b). Subsequently, a desi variety ICC 4958 genome draft was reported (Jain et al. 2013). Next-generation genome sequencing techniques and third-generation sequencing advancements have been employed for several chickpea trait improvement programs. Several genes and QTLs were mapped with refined intervals in chickpea using the advanced genomic tools and techniques such as WGRS, GBS, Restriction site-associated DNA sequencing (Rad-Seq), RNA sequencing (RNA-Seq), high-density SNP arrays, bulk segregant analysis RNA-Seq (BSR-Seq), QTL-Seq, MiRNA-Seq, Mut-Map (Manchikatla et al. 2021), Mut-Map+, MutMap-Gap and MutChromeSeq and exome sequencing including RenSeq, MutRenSeq, SMART-RenSeq and AgRenSeq, Hi-C and nanopore (Fig. 2.2). Also, improved mapping methods and techniques including GS, haplotype-based breeding, forward breeding, pan-genome, and super pangenome and integrative omics or panomics approach have been used. Over the last three decades, numerous transcriptomic studies have been performed in chickpea under abiotic stress conditions. The basic idea for these studies is mainly a comparative approach using tolerant and susceptible genotypes to get insights on the mechanism of tolerance to abiotic stresses in chickpea. The technical details of these methods were described elsewhere (Varshney et al. 2018; Jaganathan et al. 2020). The detailed information about different abiotic stresses in chickpea, efforts made by scientists through conventional and, advanced techniques of molecular biology and plant breeding for their management are provided as below.

2.4 Application of Transgenic Technology to Combat Abiotic Stresses in Chickpea

The transgenic approach remains to be the potential technology for developing stress tolerance crops without losing the yield. Several studies have been reported in chickpea for abiotic stress management using transgenic technology. To develop drought-tolerant chickpea genotypes, an osmo-regulatory gene *P5CSF129A* encoding the mutagenized Δ1-pyrroline-5-carboxylate synthetase (P5CS) for the

overproduction of proline was over expressed (Bhatnagar-Mathur et al. 2009). This study reported enhanced proline level and a modest increase in transpiration efficiency to combat drought stress in chickpea. Heterologous expression of the same gene was found to enhance salt tolerance in chickpea without affecting yield (Ghanti et al. 2011). *Agrobacterium tumefaciens* mediated transformation of *Vigna* Δ1-pyrroline-5-carboxylate synthetase (P5CS) cDNA was transferred to chickpea cultivar Annigeri and the T1 plants showed normal viable seed set under 250 mM NaCl without affecting the plant yield. Myo-Inositol monophosphatase (IMP) is an essential enzyme for many metabolic and signaling pathways in plants. Saxena et al. (2013) have reported cloning and sequencing of a full-length IMP cDNA (CaIMP). IMP activity was observed in all organs in chickpea, especially IMP level was enhanced during environmental stresses. Transcript analysis revealed that CaIMP is differentially expressed and regulated in different organs, stresses, and phytohormones. Arabidopsis transgenic plants overexpressing CaIMP exhibited improved tolerance to stress during seed germination and seedling growth, CaIMP links various metabolic pathways and plays an important role in improving seed germination and seedling growth, particularly under stressful environments.

Plant-specific NAC TFs play important roles in the regulation of various biological processes at different plant growth stages. Van Ha et al. (2014) studied the NAC TF family of chickpea and assessed the expression profiles during plant development and under dehydration and ABA treatments in a systematic manner. This study reported 71 *CaNAC* genes from the chickpea genome and presented a comprehensive expression atlas of *CaNACs* in various tissues at different developmental stages. A total of 19 *CaNACs* were found to be dehydration-responsive in chickpea roots and/or leaves in either ABA-dependent or -independent pathway. These candidate genes can be explored for developing transgenic chickpea varieties with improved productivity under drought. From its first demonstration in 2012, genome editing remains to be a promising tool for crop improvement. So far there are no reports available on genome editing in chickpea, however, there is wide scope for the researchers to explore this robust tool. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based breeding can be applied to combat abiotic stress in chickpea by utilizing the reported genes/QTLs for specific traits. With the advent techniques in genome editing including Cas9 orthologs, modifications, base editing, and prime editing, etc., this research area has the potential to revolutionize the various research field including agriculture and medicine.

2.5 Chickpea Molecular Breeding Lines Released Using Genomic Tools

The advances made in genomics using modern sequencing tools and multi-omics approaches has directly led to the development of superior varieties for abiotic stress tolerance. With the availability of QTLs and genes responsible for drought,

heat, and salinity tolerance, it is possible to introgress them into elite cultivars and develop improved varieties for single or multi stress tolerance. For instance, resequencing of 429 chickpea accessions from 45 countries identified putative candidate genes for drought and heat and these genes could be introgressed into leading chickpea varieties for abiotic stress tolerance (Varshney et al. 2019b). Pusa (BMG) 10216 is one of the best examples for the application of marker-assisted selection in developing superior chickpea varieties. Pusa 10216 is a drought-tolerant variety developed by introgressing the tolerant genes from ICC 4958 into Pusa 372, and this variety not only showed drought tolerance but also a yield improvement upto 16% (Bharadwaj et al. 2020). This variety is the product of collaborative work between national partners in India including ICRISAT and IARI in an period of only four years (https://icar.org.in/content/development-two-superior-chickpea-varieties-genomics-assisted-breeding). In another study, introgression of "*QTL-hotspot*" from ICC 4958 into a leading chickpea variety JG11 was carried out and the improved lines showed enhanced root characteristics and drought tolerance (Varshney et al. 2013a). Diversity studies and identification of candidate genes/loci for stress-related traits will allow breeders to introgressgenes/QTLs and develop abiotic stress-tolerant chickpea varieties. To characterize the variability in root architecture, a semi-hydroponic phenotyping system was applied to assess root trait variability across 270 chickpea genotypes (Chen et al. 2017). The germplasm characterized in this study could be validated and used for gene mapping. Such identified genes would allow marker-assisted breeding of the germplasm with root traits for improved adaptation to drought and other specific environments. Another key study on drought tolerance mechanism among desi and kabuli identified that desi chickpea types tolerate drought stress better than kabuli types due to better germination metabolism and trehalose accumulation, which prevented oxidative damage, helped with efficient water use, and sustained plant growth (Farooq et al. 2018). Analyzing chickpea genotypes having contrasting stress tolerance for drought, heat and cold revealed the cross-tolerance in chickpea. It was observed that tolerant genotypes selected for aspecific abiotic stress tolerance can also tolerate other abiotic stresses, resulting in high yields (Pushpavalli et al. 2020).

2.6 Application of Novel Breeding Approaches for Accelerating Abiotic Stress Tolerance in Chickpea

2.6.1 Multi-parent Populations

The novel multi-parent advanced generation inter-cross (MAGIC) populations were developed which carry QTLs for drought and heat tolerance. Such populations in chickpea are expected to have better genetic gain. Such next-generation populations are composed of 4–20 parents with higher genetic variability. MAGIC populations can be used for linkage and association mapping studies. It is helpful to obtain the

desired mapping resolution. Development of these populations allow more number of recombinations because of intercrossing several times. Further, they can be used directly or indirectly to improve tolerance to abiotic stresses. MAGIC populations have developed in Arabidopsis (Scarcelli et al. 2007), indica and japonica ecotypes of rice (Bandillo et al. 2013). At ICRISAT a multi-parent chickpea population is under development which includes eight drought tolerant genotypes (ICC 4958, ICCV 10, JAKI 9218, JG11, JG130, JG16, ICCV 97105 and ICCV 00108) from Kenya, India, and Ethiopia. These populations can serve as a potential source of variation in drought stress breeding and can be used for the fine mapping of drought tolerance related genes (Thudi et al. 2014a).

Multi-parent populations (MPPs) also include nested association mapping (NAM) where a recurrent line is crossed with a series of other alternative founder lines. Both MAGIC and NAM populations are available for major crops. NAMs are available in barley (*Hordeumvulgare*), bread wheat (*Triticumaestivum*), durum wheat (*T. durum*), maize, canola (*Brassica napus*), peanut (*Arachishypogaea*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), and soybean (*Glycine max*) (Scott et al. 2020). Bandillo et al. (2013) were able to develop the most advanced MAGIC population for indica rice via the single seed descent (SSD) method which finally consisted of 1328 lines. This combination of population subset was the source of genes to blight and blast resistance, salinity and submergence tolerance and grain quality. Bandillo et al. (2013) recorded major QTLs for submergence tolerance (*Sub1*) and *Xa4*, *Xa5* for bacterial blight resistance. When we go through the literature for the role of MPPs in crop improvement it is evident that until now most gene/QTL identification has happened in agronomic traits and biofortification. However, there few examples of abiotic stress tolerance that make MPPs a promising approach for abiotic stress breeding. A MAGIC population consisting of 11 founder lines was developed in faba bean (*Viciafaba* L.) to mine QTLs for frost tolerance. They conducted association mapping studies with 156 SNPs, the genotypes showed good variation and heritability for frost tolerance (Sallam and Martsch 2015). A similar study was done to identify QTLs for cold tolerance in maize (*Zea mays*) grown in temperate regions. Yi et al. (2020) examined 406 RIls of maize MAGIC population. Through association mapping, 858 SNPs were identified for cold tolerance and early maturity. Such revelations encourage the adoption of MAGIC populations as an efficient breeding method for identifying QTLs for abiotic stress in chickpea too. Overall, the MMPs are manmade breeding lines of a crop species but with a combination of traits of interest attained from elite genotypes. A MAGIC population can serve indirectly in developing a tolerant variety and directly as a permanent mapping population for QTL mapping. Multi-parent mapping populations have great potential for chickpea abiotic stress breeding.

2.6.2 Speed Breeding

Chickpea is a quantitative long-day plant. The rapid generation advance (RGA) or speed breeding is a technique to shorten the generation cycle of the crop of interest and fasten the breeding pipeline. Speed breeding has been found to have good potential in major crop improvement programs. A total of three to six generations per year have been achieved in different crop species such as spring wheat (*Triticumaestivum*), durum wheat (*T. durum*), barley (*Hordeumvulgare*), chickpea (*Cicer arietinum*), pea (*Pisumsativum*), and canola (*Brassica napus*) (Watson et al. 2018). Integration of speed breeding with genotyping, genome editing, and GS can fasten the abiotic stress breeding process in chickpea.

One of the earliest examples of speed breeding was published by Hickey et al. (2009) in wheat breeding at the University of Queensland with a control environment condition and SSD method for the selection of prime traits. Conventional improvement programs in peanut take 10–15 years of release time. For the first time, O'Connor et al. (2013) adopted speed breeding in a peanut line (cv. P27) under controlled environmental conditions, prolonged photoperiod, and SSD. This reduced generation time in full-season maturity varieties of peanut to 89 days and up to F_4 generation within 12 months. Traditional breeding takes up to six generations to reach homozygosity in RILs. To overcome this problem in field pea, rapid generation technology (RGT) was adopted for crosses between CDC Dakota and CDC Amarillo. With controlled photoperiod, light intensity, scheduled fertigation, and best hormonal combination application the group was able to achieve a single breeding cycle in 30–45 days, quicker than conventional SSD techniques. The research team was able to advance their breeding objectives with 5.6 generations a year using smaller amount of greenhouse space (Mobini and Warkentin 2016). This technology was not successful in the case of grain legumes. Even in chickpea to produce homozygous lines it will take 7–9 years after hybridization. Recently, (Samineni et al. 2020) proposed a rapid generation advance in chickpea. The experiment was conducted in early (JG11 & JG14), medium (ICCV 10 & JG16) and late (CDC Frontier & C235) maturity types. With extended photoperiod conditions in the greenhouse they able to induce early flowering and germination of immature seeds. The generation cycle was reduced to 43–60, 44–60, and 52–79 days and the total number of generations per year was reduced to 7.0, 6.2, and 6.0 in early, medium and late maturity types, respectively. Moreover, using the RGA protocol a RIL population of 220 lines (F_7) was also developed in chickpea which is being evaluated for nodulation traits. With exception to field experiments and screening selections, other generation advancements can be done by speed breeding. For example, if drought-tolerant genes are identified in chickpea introgression of such gene to a cultigen and homozygosity in such lines can be reached faster.

2.7 Emerging Stresses in the Context of Climate Change

The global climate is changing drastically and an unprecedented rise in atmospheric CO_2 caused by fossil fuel combustion and deforestation could lead to increases in atmospheric CO_2 concentration to ~550 ppm by mid-century and upto 1000 μmol mol-1 by the end of this century (Change 2014). Thus, increased CO_2 concentration will profoundly influence the physiological parameters caused by drastic alteration in the rise of primary photosynthates of plants. Although there has been reports of "fertilization" effect, increases in photosynthesis and stomatal conductance in response to elevated CO_2, this is often highly compromised by decreased biomass and grain production in crop plants (Battisti 2009). Increased levels of CO_2 also affect carbon partitioning in plants which in turn affects overall plant health and stress mechanisms (Rai et al. 2016). Thus new abiotic stress associated with elevated CO_2 concentration will be the emerging constraint to chickpea productivity. Also, it is believed that under nutrient-poor cropping systems, CO_2 fertilization may reduce the nutritional quality of crops through reduced nitrate assimilation and lower protein concentrations in harvestable products (Taub et al. 2008). Recently in chickpea, Palit et al. (2020) reported alterations in porphyrin and chlorophyll metabolism and other secondary metabolite synthesis pathways were observed under elevated CO_2 concentrations. Also, altered expression dynamics of stress-related major TF families like HSP 83, HSP 90, AP2, MYB and MYB-related TF families were reported. As a result, there is the need to understand the responses of chickpea to elevated CO_2 by studying the physiological and molecular changes associated with elevated CO_2. However, further research in this direction is required to understand variety-specific, stage-specific and stress response-specific response of plant under elevated CO_2 concentrations in order to develop climate resilient cultivars.

2.8 Conclusions and Future Perspectives

Global climate is changing drastically and is expected to accelerate the occurrence and severity of drought, heat, cold, etc. and limits chickpea productivity by affecting growth and development. Both physiological and biochemical traits are affected by abiotic stresses. Reproductive growth period is the most important stage affected by the drought, heat, cold and salinity resulting in substantial reductions in crop yield. Plants show a wide range of responses to these stresses by a variety of alterations in the growth and morphology. Exploring the genetic basis underlying these mechanisms will help to understand the response of plants under different abiotic stresses and to therefore optimize plant growth and development under abiotic stress conditions. Development of chickpea genomic resources and advances in genomics has greatly helped in understanding molecular basis of tolerance to different abiotic stresses. Breeders are constantly using various selection criteria to identify cultivars tolerant

to various abiotic stresses As a result, varieties tolerant to abiotic stresses have been developed and released in chickpea. QTLs for several drought tolerance related traits have been identified and "*QTL-hotspot*" region harboring root and shoor related traits has been successfully introgressed into popular cultivars and new varieties have been released for commercial cultivation. Similarly, QTLs for heat, cold and salinity tolerance traits have been identified in chickpea that can be targeted for introgression breeding. The effects of multiple stresses on physiological and biochemical traits needs be explored. In this context, the information on cross tolerance for multiple stresses is beginning to accumulate. However, more research is required to better understand the relationships among agronomically important traits and yield under multiple stress conditions. In the future, there is wide scope for the application of CRISPR/Cas9 mediated genome editing tool by utilizing the reported genes/QTLs for specific traits to develop abiotic stress tolerant cultivars in chickpea.

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Chapter 3
Genomic Designing for Abiotic Stress Tolerance in Pea (*Pisum Sativum* L.)

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Abstract *Pisum sativum* L. is a winter season legume plant belonging to the Fabaceae family. It is cultivated in more than 95 countries primarily for its protein-rich seeds for food and feed purpose. Anthropogenic climate change has shown detrimental effects on field pea production because to many biotic as well as abiotic stresses. This chapter addresses extent and impact of abiotic stresses mainly heat, water scarcity, waterlogging, frost, saline soil, and soil nutrient deficiencies. Also, we discuss their management through genetic options for the development of climate resilient pea. To achieve this, the utilization of all gene pools (primary, secondary, and tertiary) of pea for genetic advancements aimed at abiotic stress tolerance has been emphasized. Various traditional breeding methodologies and recently developed technologies like, genome wide association mapping, genomic selection, gene editing, marker assisted breeding, and nano-biotechnology have been discussed for development of abiotic stress resilient cultivars. TILLING technology can be used

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to detect mutants from physical or chemical mutagenized populations of pea. As plant developed using this method is considered as non-GMO, it has wider public acceptance. Through the identification of molecular markers and genome-wide association studies, major quantitative trait loci associated with different abiotic stress tolerances have been identified which can be helpful to detect candidate genes. The recent accessibility of the pea draft genome can help to emphasize the molecular basis of agronomically essential traits. The development of transgenic abiotic stress resistant pea and the use of virus induced gene silencing (VIGS), as well as prospect of cisgenesis have also been reviewed. Bioinformatics tools like gene and genome database with comparative gene expression database provide necessary information about abiotic stress resistant candidate genes while protein and metabolome database give information about functional protein and pathways of genes. Brief accounts on social, political and regulatory issues are also discussed. This book chapter provides all information about pea with detailed genome sequence information and major innovation in various molecular technologies to develop abiotic stress resistant pea cultivars.

Keywords Abiotic stress · Drought tolerance · Salinity · Waterlogging · Genomics · Breeding · Cold stress

3.1 Introduction

Pea belongs to the family Fabaceae (Schaefer et al. 2012), and is predominantly grown because of its seeds are protein rich. About 14.2 million metric tons per annum are produced globally for the dry seeds (FAOSTAT 2019). The top five pea producing countries contribute 70% to the global output with major shares from Canada (30%), Russia (17%), China (10%), USA (7%), and India (6%). Owing to the excellent availability, higher productivity and low-cost production, it is widely used as a commercial crop for protein production (Sun and Arntfield 2012). Pea is an important source of proteins (22.5%), calcium (64 mg/100 g), carbohydrates (62.1%), and iron (4.8 mg/100 g). In order to sustain world population by 2050 i.e. almost 10 billion people, crop productivity will have to translate its yield potential in farmers' fields (De Martinis et al. 2020).

As per 25th World Meteorological Organization report of 2018 ("Statement on the State of the Global Climate"), the duration of 2015 to 2018 were four warmest years due to which crop productivity has been constrained by biotic as well as abiotic stresses. Frequency and intensity of drought and high temperature with the manifestation of adverse climate like high rainfall, flooding, and extra tropical hurricanes, are expected to pose a serious challenge for agriculture in upcoming years (Araújo et al. 2020). Anthropogenic global warming has shown detrimental effects on cool-season legume crops. They suffer yield losses from varying biotic and abiotic stresses between 30 and 100%, depending on the degree of their intensity and frequency. The yield damages because of various abiotic stresses in legumes are given in

Table 3.1 Abiotic stress effect on production loss in major pulse crops

Plant	Abiotic stress	Percentage production loss
Field pea	Drought	21 to 54
Chick-pea	Drought	30 to 60
	pH < 6.0	20 to50
	Saline soil	Up to 50
Lentils	Drought	6 to 54
	Saline soil	Up to 50
	pH < 6.0	30 to 86
Faba-bean	Drought	21to 54

Source Rana et al. (2016b), Hidangmayum et al. (2018)

Table 3.1 (Rana et al. 2016a; Hidangmayum et al. 2018). Crops, specially legumes, are essential components of SPAC (soil–plant–atmosphere continuum) and are majorly affected by environmental factors. The abiotic stresses like extreme heat, high salt, and less water, etc., globally inflict 70% yield losses (Mahajan and Tuteja 2005). Historically, peas are grown in cold climate, however, its production are increasing in drier and warmer regions from the past two decades. However, plant growth and productivity are affected by climate change which has major impact on the accumulation of minerals and protein in legumes including pea (Soares et al. 2019). In pea, 21–54% yield losses occur from abiotic stresses (Hidangmayum et al. 2018). Many plant lifecycle processes from germination to maturity are biochemically and physiologically affected by these abiotic stresses. To fulfill the increasing demand of rising population, adaptation of crops to abiotic stresses is essential for increasing productivity (Liu et al. 2014).

Past breeding efforts have been successful in enhancing the plant ability to acclimatize the stress conditions. However, the process of developing a new variety through conventional breeding methods takes more than a decade depending upon the crop duration as it requires many cycles of crossing, testing and selection (Ahmar et al. 2020). This is all about fixing the superior alleles in right agronomic background after each cycle of selection. The key drivers are the precision, time, and the cost. It also requires application of constant monitoring of the individuals using non-invasive screening methods as well as sequencing of the genotypes to facilitate right selections. In most cases, agronomic value of the crop, its genome structure, and the form of stress factors identified and the variable environmental conditions influence the choice of appropriate breeding strategies (Yumurtaci 2015). Thus, effective methods of screening and use of available genetic diversity which will improve breeding outcomes and selection efficiency are essential. There are many challenges facing breeders, agronomists, and producers to increase world pea production. For pea to be a climate smart choice, the incremental genetic gains over each selection cycle must be maintained to remain. Pea production in Western Europe has decreased over the last twenty years, because farmers concentrated on high productive crops like canola and winter wheat. Mendel's work is the foundation for classical breeding as well as

for molecular breeding such as GWAS (genome wide association studies) as well as GS (genomic selections) techniques (Smýkal et al. 2020). Recent accessibility of the pea draft genome will further emphasize the molecular basis of agronomically vital traits (Kreplak et al. 2019). In this chapter, development of climate smart pea by utilizing their wild cultivars and landraces through plant breeding and recent genetic approaches are addressed for managing various abiotic stresses.

3.2 Key Abiotic Stresses

Abiotic stresses can alter the plant's development and reproduction due to consequence of non-biological influences majorly nutrition and environment factors. Subsequently, plants need help to restore normal conditions to reduce the harmful effects (Shao et al. 2009). Peas are usually grown as a cold climate crop in temperate regions where major abiotic stress that affects the crop is heat followed by frost, drought, salinity, and soil pH. Figure 3.1 describes the consequences of abiotic stresses and the responses of pea crop.

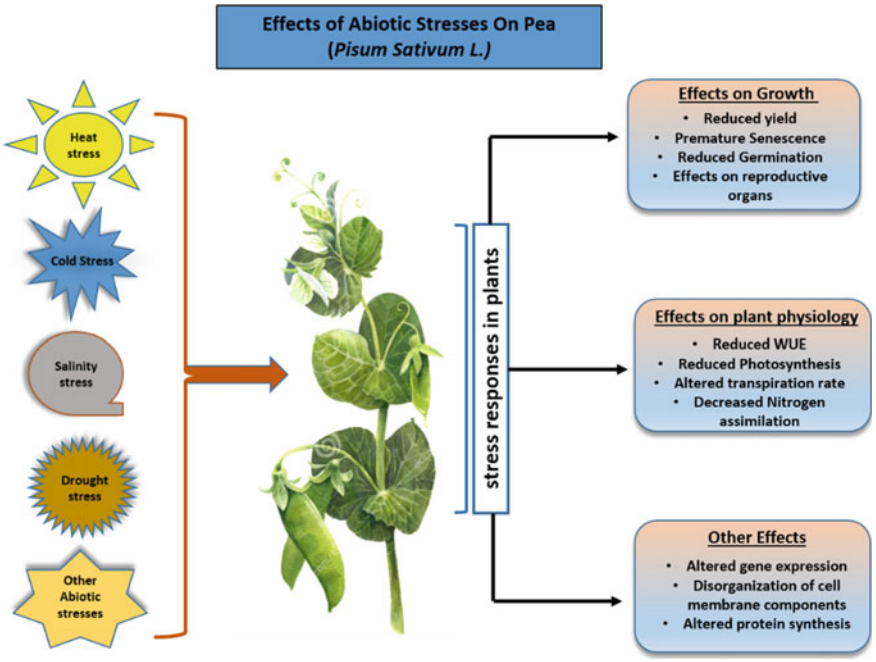

Fig. 3.1 Consequences of various abiotic stresses on *Pisum sativum* L

3.2.1 Heat Stress

The anthropogenic climate change with associated higher temperatures results in crops heat stress that leads to reduction of yield in pea. Cultivars with enhanced pod and seed preservation can mitigate the stress (Jiang et al. 2020). Ridge and Pye described that the pea production reduced by 600 kg per hector with a response to per degree increase of temperature during flowering (Ridge and Pye 1985), mainly due to reduced number of pods and seeds, and forced maturity (Guilioni et al. 2003). The regenerative organs like young pods, flower buds and flowers are most affected in heat-stressed pea (Guilioni et al. 1997). As it majorly affects reproductive organs, the crop lifecycle is accelerated under heat stress with changes in the seed development depending on ovule positions in pea pods. Overall, it produces abortive seeds at the basal ovule position due to failed fertilization in heat stress. Seed germination is fundamentally temperature dependent which largely affects germination, abnormal seedlings and abridged radical and plumule growth of various legume crops (Hasanuzzaman et al. 2013). The feminine gametophyte is less prone to heat stress compared to the masculine gametophyte (Kaushal et al. 2013, 2016). High temperature stress attributes to lower seed set in legume crops such as pea (Jiang et al. 2015), chickpea (Devasirvatham et al. 2012), and lentil (Kumari et al. 2018) due to sterile pollens. High temperatures also probably inhibit the length of style and subsequently increase abnormalities in the growth of ovaries (Srinivasan et al. 1999). In the crops of Fabaceae family, the chlorinated form of auxin, 4-chloroindole-3-acetic acid that regulates reproductive stage (Reinecke 1999; Ozga et al. 2017). This is majorly affected by heat stress due to alteration in signaling of auxin biosynthesis in emerging anthers, and cause pollen anomalies (Higashitani 2013; Ozga et al. 2017). It is also reported that the adverse heat-stress effects on enlargement, partition and separation in egg and synergids of female gametophyte in beans (Sage et al. 2015).

'Omics' tools like transcriptomics, proteomics, metabolomics and obviously genomics have modernized research in plant science and studies (Yuan et al. 2008). Plant proteome and metabolome compositions are strongly affected by stresses because of alteration in gene expression. Therefore, omics technology is essential to understand plant stress tolerance (Ramalingam et al. 2015). In legumes, research are done for transcriptomic analysis intended for heat tolerance. Various thermo tolerant gene expression analyzed by cDNA—AFLP (amplified fragment length polymorphism) technique in cow pea (Simões-Araújo et al. 2002), 25 candidate heat shock factors (HSF)- ESTs (expressed sequence tags) in soybean, 21 in *Medicago truncatula* and 19 in *Lotus japonicas* were identified (Soares-Cavalcanti et al. 2012). In faba bean, the transcript expression of *VfHsp17.9CII* gene showing substantial (620-fold) alteration when exposed to elevated temperature exposure are cloned (Kumar et al. 2015). In pea, the consequences of major abiotic stress on pea's mitochondrial proteome is studies with the help of transcriptome with combination with physiological measurements (Taylor et al. 2005; Zhao et al. 2020).

In order to accomplish and improved depiction of applicant genes in transcriptome sequence, next-generation sequencing (NGS) technology was used on ICC4958

chickpea genotype and *DNAJ, HSP70*, as well as *HSP91* genes were recognized using Illumina sequencing (Hiremath et al. 2011; Martin et al. 2013). These advanced approaches along with genomics knowledge precisely identify candidate genes accountable for significant traits which are invaluable for legume breeding plans (Langridge and Fleury 2011).

3.2.2 Cold Stress

Cold stress is a major abiotic stress for worldwide agricultural production that mainly affects cool season food legumes. These crops are highly subtle to freezing temperature, especially during seedling, blossoming, initial pod forming as well as seed filling phases (Siddique et al. 1999). Pea has insufficient resistance to frost conditions (Stoddard et al. 2006), including to radiant frost (Shafiq et al. 2012). Plant tolerance vary according to physiologically different chilling (0–15 °C) as well as freezing temperature. Acclimatization is supplemented by surges in homogalacturon, xylogalacturon and extremely branched Rhamnogalacturonan I with (15)-α-arabinans and (14)-β-galactans, indicating the role of esterified pectins (Baldwin et al. 2014). It is reported that to acclimatize cold-stress, pea accumulates starch and, depending on the cold and light conditions, decreases the water content in root and shoot in tolerant plants, during exposure (Bourion et al. 2003). Pea plant also accumulates carbohydrate at low temperature (Streb et al. 2003). The 32 proteins related with frost tolerance were recognized in frost tolerant pea line 'Champagne', which withstands frost by reorienting the energy metabolism (Dumont et al. 2011). For cold acclimatization, transcription factors C-repeat Binding Factor/Dehydration Responsive Element Binding is a significant element in plants.

3.2.3 Drought Stress

Drought at any growth stage causes serious damage to crop performance. Ability of a plant to reproduce and survive in water deficient conditions without affecting yield is the best strategy to manage drought (Fleury et al. 2010). In pea, drought mainly modifies protein and starch ratios at flowering and pod filling stages which negatively affect its yield (Baigorri et al. 1999; Červenski et al. 2017). Drought in combination with high temperature has combined effects on plant physiology, leading to severe yield reduction (Pandey et al. 2017). In order to conserve water, plants suppress stomatal opening which reduces the photosynthetic efficiency, with complete stomata closure at 60% relative soil water content (Moisa et al. 2019; Nabi et al. 2019). Two important osmoprotectants i.e., proline and total soluble sugars, which help reduce cell injury and damage with reduction of relative water content in pea are accumulated (Garg et al. 2018). It was reported that the pea G protein

provides tolerance to heat in transgenic tobacco plants by regulating nitric oxide-induced stomatal movements (Bhardwaj et al. 2020). This stomatal movement is regulated by COP1 with respect to dehydration (Moazzam-Jazi et al. 2018). The wild pea genotype HR1 adapted to drought environment was studied in field conditions (Iglesias-Garcia et al. 2017). It was found that the loss of water content strongly accumulated ROS and lipid peroxidation. Also DREB2A related gene expression was induced with two-fold higher in roots and 60% higher in leaves after 10 days of dehydration (Jovanović et al. 2013). For biomass, water deficiency as well as salt tolerance in *Medicago truncatula*, 11 Tnt1 retrotransposon (the transposable element of tobacco cell type 1) tagged mutant populations were screened and it was revealed that Tnt1 insertion in the *SPL8* gene caused mutation. Based on *SPL8* sequence of *Medicago*, transgenic alfalfa *SPL8* (*MsSPL8*) plants were produced in which *MsSPL8* down-regulated transgenic plants revealed significant enhancement on drought as well as salt tolerance by regulating various anthocyanin genes such as *CHS*, *PAP1* and *DFR* which are closely associated with abiotic stresses response. It also showed up to 43% and 86% increased biomass in first and second harvests, respectively (Gou et al. 2018).

A total of 11 and seven nodule proteins controlled by drought stress in pea and *Rhizobium leguminosarum* genomes were analyzed and from these, three proteins related to flavonoid metabolism, three RNA-binding proteins and two to sulfur metabolism were identified. These can be molecular targets for enhancing drought tolerance in legumes (Irar et al. 2014). To mitigate detrimental effects of drought in legumes, plant growth-promoting rhizhobacteria (PGPR) become an effective practice (Khan et al. 2019). It has been found that the deaminase-producing 1-aminocyclopropane-1-carboxylate (ACC) PGPR provides drought tolerance by controlling ethylene levels in plants. The influence of three ACC-deaminase generating rhizobacteria—*Ochrobactrumpseudogrignonense* RJ12, *Pseudomonas* sp.RJ15 and *Bacillus subtilisRJ46* on water deficiency stress mitigation mung bean and pea showed significant increase in shoot and root length, and seed germination percentage (Saikia et al. 2018). Nadeem et al. (2019) studied the progress on the examination of water deficiency stress in legume plants including pea. Using classical genetics, molecular breeding and the draft genome, high-throughput marker development for performing GWAS for identification of novel genomic variants related to drought tolerance is facilitated. Additionally, developing 'omics' sciences, comprising proteomics, genomics and metabolomics with innovative techniques such as genome editing tools and 'speed breeding' will efficiently increase the development of drought tolerant pea cultivars (Nadeem et al. 2019). The basis of drought tolerance can also be analyzed in crop wild relatives (CWR) of pea for characters such as leaf waxiness obtained in *P. sativum*, whereas, *P. fulvum* showed less drought susceptibility (Naim-Feil et al. 2017).

Root system architectures (RSA) play vital role in mitigating various abiotic stresses in plants. In the root system of dicotyledonous plants like *Arabidopsis* and legumes such as *Medicago* as well as pea forms primary root with continuous order of lateral roots (Sorin et al. 2005; Rich and Watt 2013). The size and thickness of primary and lateral roots are important for water deficiency stress tolerance. In rice,

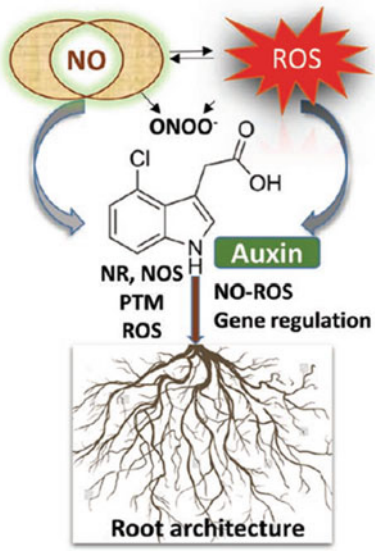

Fig. 3.2 Signaling molecules that affect root system architecture (Prakash et al. 2020)

there is growth of excessive root length density with enlarged root diameter with respect to water deficiency stress (Abd Allah et al. 2010). In maize, plants with strong root length density and lateral roots have elevated plant water levels which improve stomatal conductivity and photosynthesis, leading to an overall increase in plant growth (Lynch et al. 2014; Zhan et al. 2015). Nitric oxide as well as reactive oxygen species plays significant role in regulation of several plant functions during root formation. Nitric oxide can increase as well as restrict RSA by moderating enzyme activities via post-translational modification (Fig. 3.2). Spatial regulations of ROS controls cell development in the primary root and acts in combination with Nitric oxide to stimulate lateral-root primordia (Prakash et al. 2020).

In dry areas, water-use-efficiency (WUE) is defined by grain yield per unit of water supplied (Yin et al. 2015). WUE is also affected by drought and salinity stress due to significantly decreased stomatal conductance, photosynthetic rate, transpiration rate, and intercellular CO_2 concentration. Atmospheric CO_2 concentrations have increased to over 400 ppm. Bourgault et al. grown pea cultivars in free air CO_2enrichment (FACE) and found yield was increased significantly (26%) because of a growth in the amount of grains from each pod, grain size, amount of pods per unit area, but there was no effect on the harvest index (Bourgault et al. 2016).The e[CO_2] also decreased the stomatal conductance and transpiration which helps to increase the WUE. This shows that better yield response is possible to elevated CO_2 in dry areas (Leakey et al. 2009).

Water stress has significant negative consequence on initial development and survival of the plant (Machado et al. 2017). For the germination process, water is the most important factor as it is basic necessity for most physiological as well as biological developments (Gouveia et al. 2017). As water deficiency increases, solute

concentration increases which lead to changes in intracellular pH and this causes degenerative reactions like loss of membrane integrity and denaturation of protein causing abnormally damaged seedlings. In pea plants, osmotic stress caused by PEG 6000, leads to increased lateral root numbers, induces a restriction of the primary root and also substantial increase of nitric oxide (Kolbert et al. 2008). Recently, Pereira et al. (2020) evaluated physiology of seeds and initial seedling development in pea with different salt and water potentials like PEG 6000, NaCl and KCl solutions. Different physiological characters and epicotyl dry matter were found to be negatively affected with induced stress (Pereira et al. 2020).

Although global warming increase draught stress in agricultural crops, plants established many schemes to mitigate oxidative stress to reinstate the redox homeostasis by plant endophytes which are their symbiotic partners (Vujanovic and Germida 2017). The fungal endophytes produce several antioxidants which suppress of antioxidant genes under draught conditions that help to overcome seed dormancy to increase early stress resistance by moderating gene regulation of phytohormones (Waqas et al. 2014). In pea, transgenerational endophyte-mediated benefits resulted in better seed germination and reduced relative oxygen levels in plant-roots as revealed by down-regulation of proline, manganese superoxide dismutase (Mn*SOD*) and *SOD* genes due to draught stress. This research indicated that endophytes can enhance pea resilience to abiotic stress (Kumari and Vujanovic 2020).

3.2.4 Salinity

Salinity is the most severe issue throughout the world which damages plants in many ways. It is estimated that the salt-affected area (sodium and saline) is near 20% of the world gross cultivable land and is increasing 10% per annum. It is also revealed that 50% of the cultivable land will be sacrificed with salinity by 2050 (Hussain et al. 2019). In pea, salinity is one of the most often observed stresses that reduce the volume of soluble water results water deficiency. Hyperosmotic as well as hyperionic pressures because of sodium and chlorine ion deposition from salty soil cause a dramatic drop in photosynthesis, and decreased intracellular ROS which majorly affects the membrane impairment and hence the cell-death (Negrão et al. 2017; Zelm et al. 2020). The detrimental effect of saline soil on fertility is noticed during development period with decreased metabolite partitioning which further leads to a higher loss of seed yield. Salinity induced oxidative stress cause impairment of biological membranes as well as decreases carboxylation rate. Elevated sodium and chloride ion toxicity in tissues leads to reduction of leaf growth and causes early leaves senescence. Salt stresses also decreases the photosynthesis rate because of decrease in stomatal conductance (Farooq et al. 2017). The effect on photosynthesis due to salt stress is described in Fig. 3.3.

In the northeast regions of India, majority of the soil are acidic (pH 5.0–6.0), which makes them extremely rich in Fe^{+} and Al and creates scarcity of P and K (Thakuria et al. 2018). For pea, the acidic soils create challenging situation for farming by

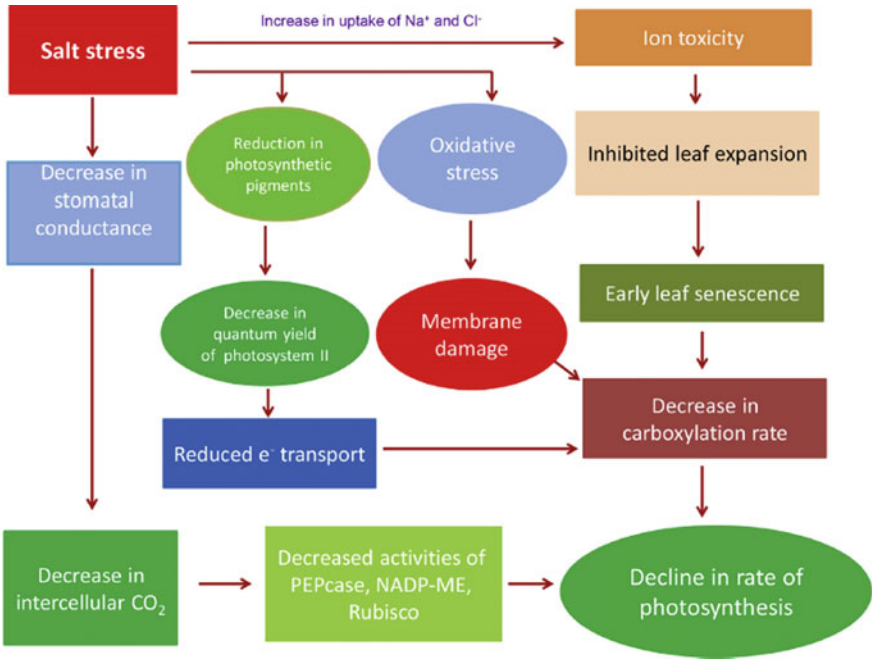

Fig. 3.3 Effect on photosynthesis due to salinity stress (Farooq et al. 2017)

creating severe toxicity because of higher availability of iron (Fe), manganese (Mn) and aluminum (Al) (Bojórquez-Quintal et al. 2017). Additionally, pea plants require relatively higher phosphorus necessities for creation of nodule and for optimal photosynthesis (Powers and Thavarajah 2019). Recently, Chaudhary et al. (2020) showed enhancement of bacterial taxonomy identified for plant growth promotion (PGP) that helps to eliminate toxic elements from rhizosphere.

In pea, a mutant population was created by the chemical mutagen, ethyl methane sulphonates (EMS), in which two mutated genes, cinnamyl alcohol dehydrogenase (*CAD*) and Dehydrins (DHNs) were identified. These plays vital role for tolerance against numerous stresses like salinity (Hameed 2018). Transgenic pea plants are good examples of enhanced salt stress tolerance, in which overexpression of the Na^+/H^+ gene from *Arabidopsis thaliana* in association with PGPR promotes the development of salt-tolerant plants. Further, the transgenic pea plants containing *AtNHX1*exhibited salt-stress tolerance in consequent generations on a span of six years (Ali et al. 2018). Gibberellic acid (GA3) has been also reported in pea to increase the percentage of germination and plantlet development under the salinity stress. As a result, seed priming with 0.2 g per liter gibberellic acid considerably improves the percent germination, duration of germination, enhanced shoot and root lengths, as well as total crop volume (Tsegay and Andargie Agriculture). Seed socking and foliar spray with *Moringa oleifera* leaf extract (MLE) and ascorbic acid (AA) shield plants with respect to salinity and improve plant growth in pea

(Mohamed De et al. 2016). Salicylic acid (SA) and nitric oxide (NO) are used as signal molecules in plant stress reactions and perform vital roles in key regulatory routes for growth, development, and metabolism. Restricting the effects of salinity on the radicle and accumulation of biomass have been effected by SA and/or sodium nitroprusside, while their combined application has been found effective in peas (Yadu et al. 2017). Proline and phytohormones play a defensive role against salinity stress. Throughout the salinity stress, osmolytes such as proline retains cell homeostasis by osmotic control and induces beneficial physiological mechanisms (Iqbal et al. 2014). The valuable role of cytochrome oxidase (COX) and alternative oxidase (AOX) for maximum photosynthetic quality in pea leaf under saline stress condition were reposted (Analin et al. 2020). Parihar et al. (2020) described that the inoculation of Arbuscular mycorrhizal fungi (AMF) in pea crop enhances tolerance to salinity by improving nutritional content, chlorophyll synthesis, and biochemical status, which greatly increased production of biomass, yield and growth. Recently in pea (cv. Meteor), four antioxidants genes. i.e., *Mn-superoxide dismutase (Mn-SOD), Peroxiredoxin (PrxIIF), Thioredoxin (Trxo1)*, and *Alternative oxidase (AOX)* were identified, which are up-regulated under salt stress conditions and can help to mitigate stress conditions. These genes may be useful for the creation of salt tolerant pea varieties (Manzoor et al. 2020).

3.2.5 Waterlogging

There are two types of waterlogging, transient and continuous, causing severe damage to crops (Shahin Uz Zaman 2019). Waterlogging can lead to the loss of soil nutrients and disrupts soil structure. Due to waterlogging, around 25 to 30% yield loss were observed in pigeonpea (Bansal and Srivastava 2017). In pea and white lupin more severe symptoms were observed like abscission of leaflets and detrimental effect on newly opened flowers. Similar patterns in different various crops like chickpea, grass pea, lentil, mungbean, pea, pigeonpea, and soybean have also been observed (Cowie 1996; Bacanamwo and Purcell 1999; Kumar et al. 2013; Malik et al. 2015). Waterlogging at the germination stage is also assessed in a pea recombinant inbred lines from a bi-parental cross of waterlogging (WL)-contrasting parents to evaluate phenotypic variations, to recognize the genetics of waterlogging tolerance, and to identify characters for indirect selection. This population was screened against both waterlogged and drained soils and assayed for testa integrity/leakage in calcium sulphate solution. The 90% plants with dark-colored testa identified as waterlogging tolerant, while light-colored testa phenotype plants were waterlogging sensitive. Thus, the color of testa and conductance indicated is interesting phenotypic selection of pea WL tolerant variety (Zaman et al. 2019b). This trait is also related with WL resistance in other crops as well (Hou and Thseng 1991; Ueno and Takahashi 1997; Zhang et al. 2008). For instance, dark testa phenotype in wheat (Ueno and Takahashi 1997) and soybean (Hou and Thseng 1991) are tolerant to waterlogging as compared to lighter (white/yellow) testa cultivars. Moreover, seeds size is also an important

factor for waterlogging. One report suggested that a smaller size of seeds have more tolerant of waterlogging as compared to a larger ones (Sayama et al. 2009). Several factors like shortage of oxygen demand and inhibition of ATP formation leads to an unbalanced cell membrane, ultimately decreasing germination (Jackson and Drew 1984; Zaman et al. 2019a). ATP formation decreases and various lipid metabolic enzymes like lipase and lipoxygenase are increased which lead to cell membrane damage (Rawyler et al. 1999). During waterlogging, nitrogen can be wiped out from the soil and the plant does not have enough amount of nitrogen. Many waterlogging tolerant pea germplasm accessions were identified in Ethiopia (Tsidu 2012; Zaman et al. 2019b).

In pea, three varieties (BM-3, NL-2, and Kaspa) were screened under waterlogging stress conditions for seven days. These three varieties, BM3, NL-2, and Kaspa showed 14%, 40%, and 55% radicle emergence, respectively. These cultivars were also screened for alteration of gene during growth with "quiescence/escape" mechanism for waterlogging tolerances. Whole-genome RNA sequence was carried out to analyze differential expressed genes. In Kaspa, strong induced tyrosine-protein kinase and suppressed fat metabolism gene (linoleate 9S-lipoxygenase 5) were observed. Contrastively, enhanced energy utilization approach was observed in NL-2 by induction of a fat metabolizing gene. In BM-3, WL non-tolerant germinating seeds was related the induction of a Kuntz-type trypsin/protease inhibitor which leads to extreme lipid absorption and membrane outflow related to WL damage. The pathway analysis by gene ontology showed that storage protein metabolism is up-regulated intolerant genotypes while down-regulated in sensitive genotype. This approach offers a base to generate waterlogging tolerant pea cultivars (Zaman et al. 2018, 2019a).

3.2.6 Nutrient Use Efficiency

The Legumes require limited amount of nitrogen fertilizers at initial stage of plant development in accordance of their capacity to fix atmospheric nitrogen (N) symbiotic relation with *Rhizobium*. However, balanced fertilization (NPK) helps legumes to tolerate abiotic stresses like drought and heat by enhancing water uptake and retention (Rana et al. 2016a). Two types of nutrients, macro and micro, are used for plant cultivation. The need of primary Macro nutrients N, P and K and secondary nutrients like Ca, Mg and S are necessary in major amount. Whereas, micronutrients are needed in smaller amounts, which includes Fe, B, Mn, and Zn, Cu, Cl and Mo. Deficiencies of these important nutrients have adverse effects on pea crop. To overcome nutrient deficiency and reduce its effect on crop yield, developing nutrient use efficient pea varieties is important. Two pea mutants *bronze* (*brz*) and *degenerate leaves* (*dgl*) have been identified with increased iron uptake (Robinson et al. 2019). To increase nutrient uptake and seed yield, the application of combined micronutrients is proven more effective than solo use of micronutrients. Field experiments showed that pea shows extreme intake of N, P, and S when it was collectively applied

with Co, B, and Mo or individually with Co (Singh et al. 2015). It is also reported that in pea plants, to fulfil nutrition its requirement and to resist stresses, plant itself modulates its rhizosphere community. A plenty of Proteobacteria identified in the rhizosphere, while the bulk-soils consist of Firmicutes. The rhizosphere indicates the propotion of *Pseudomonas*, *Nitrobacter*, *Sphingomonas*, *Pantoea, Enterobacter,* and *Rhizobium* were considerably higher at the genus level (Chaudhari et al. 2020).

3.3 Genetic Resources

Pea is known as ancient, domesticated crop globally and there are around 98 thousand accessions preserved worldwide. The top 25 pea collections hold around 72 thousand accessions together while remaining 27 thousand accessions are scattered globally in more than 146 collections (Smýkal et al. 2013). Recently, the major pea germplasms are conserved by INRAE, France, which hold around 8,839 accessions and over 9,000 TILLING mutant lines. Many national institutes have pea germplasm collections such as, 7,432 accessions by the Australian Grains Gene bank, 8,203 by the Vavilov Institute (Russia), 6,827 by USDA (USA), 6,105 by ICARDA, 5,343 by the Leibniz Institute of Plant Genetics and Crop Plant Research (Germany), 4,558 by the Instituto Di Genetica Vegetale Italy, 3,837 by the Institute of Crop Science (China), 3,609 by National Bureau of Plant Genetic Resources (India) and 3,006 accessions by the John Innes Centre, UK (Coyne et al. 2020). The main gene banks consisting pea germplasms described in Table 3.2 (Smýkal et al. 2012).

3.3.1 Primary and Secondary Gene Pool

In this gene pool, pea subspecies *sativum* involves var. *sativum* and var. as well as *arvense,* and while subspecies *elatius* involves var. *elatius*, var. *pumilio as well as* var. *brevipedunculatum.* Due to ample genomic variance and crossing these pools is foremost source to develop climate resilient varieties afor various abiotic stresses. In contrast to primary, secondary pools have low compatibility for crossing as well as inferior fertility broaden area to additional species, *P. fulvum*and and *P. abyssinicum.* (Trněný et al. 2018; Weeden 2018). These two create distinct adjacent subdivisions in which, a subsection of *P. sativum* subsp. *elatius* located in-between them. In few research, *P. abyssinicum* is placed in middle of of *P. sativum* subsp. *elatius* and *P. fulvum* and also revealed that it has very low genetic diversity (Smýkal et al. 2011, 2013). *P. fulvum* may represent a pool of genes that could be used in pea to enhance salinity and water deficiency resistance (Naim-Feil et al. 2017).

Table 3.2 List global pea germplasm collection institutes (Smýkal et al. 2012)

Code	Country	Institute	No. of accessions	Genotyped	Phenotyped	Core
VIR	Russia	N.I. Vavilov Research Institute of Plant Industry, St. Petersburg	6,790	Not	Not	
USDA	USA	Plant Germplasm Introduction and Testing Research Station, Pullman	5,400	Yes	Yes	Formed
BAR	Italy	Istituto del Germoplasma, Bari	4,297	Not	Not	
SAD	Bulgaria	Institute of Plant Introduction and Genetic Resources, Sadovo	2,787	Not	Not	
NGB	Sweden	NordGen, Nordic Genetic Resource Centre, Alnarp	2,724	Not	Not	
CGN	The Netherlands	Centre for Genetic Resources, Wageningen	1,008	Not	Not	
ATFC	Australia	Australian Temperate Field Crop Collection, Horsham	6,567	Yes	Yes	Formed
ICARDA	Syria	International Center for Agricultural Research in the Dry Areas, Aleppo	6,105	Not	Not	
GAT	Germany	Leibniz Institute of Plant Genetics and Crop Plant Research, Gaterleben	5,336	Not	Not	

(continued)

Table 3.2 (continued)

Code	Country	Institute	No. of accessions	Genotyped	Phenotyped	Core
ICAR	China	Institute of Crop Sciences, CAAS China	3,837	Partly	Not	
JIC	UK	John Innes Centre, Norwich	3,557	Yes	Yes	Formed
WTD	Poland	Plant Breeding and Acclimatization Institute Blonie, Radzikow	2,899	Not	Not	
INRA	France	INRA CRG Légumineuse à grosses graines, Dijon	1,891	Partly	Yes	Formed
UKR	Ukraine	Yurjev Institute of Plant Breeding, Kharkov	1,671	Not	Not	
CZE	Czech Republic	AGRITEC, Research, Breeding and Services Ltd., Sumperk	1,284	Yes	Yes	Formed
HUN	Hungary	Institute for Agrobotany, Tapioszele	1,188	Not	Not	

3.3.2 Tertiary Gene Pool

This pool consists of *Vavilovia* and *Lathyrus* which have a close phylogenetic relationship with peas (Smýkal et al. 2011). *P. vavilovia* is restricted to high elevations in the Caucasus (Nair 2019). Many biotechnological approaches have been developed for in vitro propagation of *P. vavilovia* which will help to accelerate the breeding and taxonomical understanding (Ochatt et al. 2016). Another species this pool is the genus *Lathyrus* which grown for over eight hundred years due to its resistance to flooding, water deficiency as well as to saline soil (Sarkar et al. 2019). The *Lathyrus* genus contains 187 species. There is about 4,200 accessions of *Lathyrus* germplasm at ICARDA, about 2,600 accessions at NBPGR in India and around 4,000 cultivars are held by CBN PMP (Vaz Patto and Rubiales 2014; Sarkar et al. 2019).

3.4 Conventional Breeding for Abiotic Stress Resistance

Since the Mendel's experiments in the mid-1800s, conventional breeding methods like pure line selection and hybridization trailed by bulk collection, pedigree selection, and their modifications have been successfully used to generate creative varieties with novel traits (Wani and Gosal 2020). Nevertheless, several characters related to abiotic stress tolerance require substantial enhancement for improved adaptation and stability under difficult growing conditions. The key objective in pea breeding is not only to enhance grain yield but also to combine tolerance to key abiotic and biotic stresses and to improve seed nutritional quality by effectively using diversity present in the pea gene pool. Water deficiency and temperature stress at flowering are foremost abiotic stresses in pea whereas, cold, saline soil, and flood in early growth periods are significant in different areas (Tayeh et al. 2015b). The Canadian programs mainly include improvement of varieties with significantly increased yield and early maturation with tolerance to powdery mildew, WL as well as mycosphaerella blight. Two public breeding programs in the USA are intensively based on generation of autumn-sown cultivars with high nutrition quality with tolerance to various diseases. In India, breeding programs are usually centered on yellow pea having seed coat without pigmentation. Current breeding goals include varieties with improved harvest index, short span pea weevil tolerance, and tolerance to terminal drought and frost (Warkentin 2015). In France, INRA commenced to develop autumn seeded cultivars as a approach to upsurge yield capacity, high biomass and prior maturity in order to mitigate water deficiency and temperature stress (Hanocq et al. 2009).

The production of cool season pea cultivars has added as improved breeding targets for winter hardiness, suitable flowering times to avoid frost initiation at the end of winter season, as well as seed filling for the period of rising temperature at the initiation of summer.

Over the two decades, several pea cultivars have been developed through conventional breeding and approximately 2% yield gain per year has been achieved. A nearly total shift in western Canada has been made from 'leafy' to 'semi-leafless' varieties having the *afila* gene cause development of tendrils replace by tendrils. The lodging score of widely grown cultivars have significantly enhance over two decades (Warkentin 2015). In the USA and Europe, winter resilient cultivars have been established, which give improved yield due to its extended growth period and increased biomass to circumvent water deficiency and temperature stress (Hanocq et al. 2009). The insertion of *Hr* gene developed winter hardiness by delayed flower generation period until completion of freezing season (Lejeune-Hénaut et al. 2008). In landraces, improved stress tolerance was established for boron harmfulness (Bagheri et al. 1994), saline soil (Leonforte et al. 2013a), iron scarcity (Kabir et al. 2012), and temperature resistance throughout flowering (Tayeh et al. 2015b). Several boron resistant cultivars were identified through phenotypic screening in field pea breeding programs (Bennett 2012). Recently, two heat tolerant cultivars with increased yield namely CDC Meadow and Naparnyk have been developed by evaluation of 16 pea cultivars under heat stress and its effect on phenology, and other components (Jiang

et al. 2020). There are several varieties are developed in India with semi-leafless and powdery mildew tolerance. Many varieties for instance, Vikas, Prakash and Adarsh with fast growing capability are developed to mitigate stress with production capacity of 2.5 tons per hector. During 1980s, in north-eastern region of India had detrimental rust effect on pea crop, so to circumvent this issue development of resistant varieties was commenced. Some rust resistance varieties for instance, HUDP 15, Plant P 42, Aman, Swati, and Prakash have been developed (Warkentin 2015).

The future targets in pea breeding include (i) development of plant morphology and phenology for innovative breeding system, (ii) refinement of pea symbiotic relationship with rhizobia, mycorrhiza and other advantageous microorganisms to observe plant resistance for abiotic stresses, (iii) alteration of seed composition and innovative end-use application possibilities.

3.5 Limitations of Conventional Breeding

Since the birth of principle of genetics in 1790s, genomic studies on peas are being conducted (Smýkal et al. 2012). Pea is among the principal plants of which mitotic karyotype and karyogram was developed. Depending upon the conventional cytogenetic rules, the definite classification for the chromosomes of pea has been carried out and chromosome pairs were ordered (Praça-Fontes et al. 2014). Although an important crop, pea has not gained much attention that delayed progress in the genomics, advancement of molecular biology, and bioinformatics methods (Bhattacharyya et al. 1990; Hofer et al. 2009), and it is also lagged behind many crops such as wheat, soybean, corn, and rice in relation of genetic resources. The genome of pea assessed at 4.45 Gb is structured into 7 pairs of chromosome ($2n = 14$) and it largely composed of transposable elements majorly with Ty3/gypsy family (Macas et al. 2007). The huge genome size and more mobile elements, lead to a delay in the availability and development of genomic tools in pea (Tayeh et al. 2015a).

Morphological features, depend upon illustration of plant traits is a natural way to detect the genetic dissimilarity and make appropriate selection of desired recombinants. These features are significant for creation of gene pools and in making resourceful use of germplasm resources (Santos et al. 2012). Morphological and biochemical markers has not been used widely as selection criteria as these are affected significantly by environmental factors. For determining genetic variation and evolutionary relationships, various molecular markers and sequencing techniques are routinely used (Ahmad et al. 2020). Recently, Guindon et al. (2019) developed a linkage map for pea by means of SSR, SNP, SRAP markers to classify QTLs related with yield-related traits. The F_2 individuals were evaluated using various molecular techniques for successful in development of set of 872 polymorphic markers to map linkages. The consequential map involves 128 genetic markers spread across 9 linkage groups (LGs), covers 655.5 cM. The span of the LGs varied between 49.1 and 114.8 cM, with 8–26 markers. The regions of gene associated with traits i.e., QTLs can be proficiently analysed by molecular markers as well as linkage maps in

pea, which provides useful information for molecular breeding and to develop useful pea cultivars (Guindon et al. 2019).

From quite some time now, traditional farming methods have reduced the genetic variety of crops. A various traditional and molecular methods have been utilized to enhance the agronomic characteristics related to yield, quality as well as tolerance to abiotic and biotic stress in plants, including mutagenic breeding, genetic selection, soma clonal variation, and whole-genome sequencing. The pea genome sequence has been recently elucidated. This will help both basic research and pea breeding efforts (Kreplak et al. 2019). Recent developments in genome editing with programmable nucleases, CRISPR/Cas have opened a new door in plant breeding (Ahmar et al. 2020). Therefore, to enhance crop production, farmers and scientists all over the world need to use new techniques such as speed breeding, high throughput phenotyping and genome editing tools. This advancement in molecular breeding will help to produce novel crop variety with desirable characters with tolerance to biotic and abiotic stress in pea.

3.6 Diversity Exploration

3.6.1 Phenotype-Based Diversity Analysis

The description of individual traits based on morphological characters are the most conventional and instinctive approach to detect genetic diversity in crop plants. Pea diversity is defined according to seed quality traits based on physicochemical functional properties of 105 accessions (Santos et al. 2012). Basic composition parameters were also considered for evaluation such as protein, fat, fiber and resistant starch for nutritional traits, seed surface, and color and seed shape for seed traits. As per to diverse morphological traits (Fig. 3.4), pea seeds were classified as elliptical, irregular cylindrical or rhomboid as seed shape, light-green, dark- green, yellow green,

Fig. 3.4 Pea diversity categorized by shape, color as well as texture (Santos et al. 2019)

orange brown, cream yellow and brown as seed color, and smooth or rough as seed surface (Santos et al. 2019).

Tianyao Zhao et al. (2020) evaluated genetic diversity of 75 pea cultivars based upon morphological characteristics (seed texture, coat pigmentation, shape, cotyledon appearance, and hundred seed weight). Apart from phenotypic characters, chemical composition of seeds were also analyzed such as entire flavonoid content, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate), as well as free radical scavenging, total phenolic-content, and ferric reducing antioxidant capacity. Peas are divided into three groups according to dendrogram resulted by hierarchical cluster analysis. Dark seeds have properties as functional food with their main role in providing dietary protein and fibre (Zhao et al. 2020). Cold tolerant pea varieties are valuable to improve cold stress for consistent yield and rise in the winter growing area in natural cold season in China. Screening of 3672 pea accessions for cold tolerance resulted in identification of genotypes with moderate resistance (835), and high resistance (214). Further evaluation of high and moderately resistant genotypes showed that the cultivars grown in cold region have more resistance than cultivars grown in spring season (Zhang et al. 2016). Based on morphological traits, variable degree of genetic relatedness among seven pea lines was revealed (Kumar et al. 2019b). Number of pods/each plant has significant genotypic as well as phenotypic correlations ($r = 0.685$ and 0.670 at $P \leq 0.01$) with seed yield followed by pod length (0.639), number of nodes to first flowering (0.576).

3.6.2 Genotype-Based Diversity Analysis

Genetic diversity is a basic tool in designing breeding strategies (Kaur et al. 2018). Many researches has been conducted by numerous technology for analysis of genetic variance in pea germplasm (Samec and Našinec 1995; Zong et al. 2008). Appropriate breeding systems can be designed for the improvement of desired characters based on the study of heritability and genetic advancement (Kumari et al. 2013). Molecular markers are majorly utilized to classify genetic interactions and to discover genetic diversity (Smýkal et al. 2008), which have impedes way to classify genotype based diversity analysis based on polymorphism due to which traditional breeding efficacy are increased. A numerous DNA markers have been utilized such as inter simple sequence repeats (ISSR) (Zietkiewicz et al. 1994), simple sequence repeats (Beckmann 1990), random amplified polymorphic markers (Williams et al. 1990), as well as amplified fragment length polymorphic markers (Vos 1995). Amongst all, SSR are extensively used for genotype based polymorphism analysis in crop species (Blair et al. 2007). Based on 18 SSR markers, seven parental lines of pea could be classified majorly into two groups. It was found that parentage and crosses have genetic variability for all the agro-morphological traits. The genotype based polymorphism study carried out from 28 pea genotypes by 32 SSR which allowed selection of Pant P-31, HUDP-15, S 143, HUDP-27, Pant P-25, HUDP-9 as parents for hybridization, which produced desirable recombinants in segregating generations

(Kumari et al. 2013). Significant alteration was observed among the genotypes of garden peas for number of branches, length of internode, plant height, distance to first node, total nodes, resulting in the identification of GEN 1, 4, 6 as well as GEN 9 as elite genotypes (Kalapchieva et al. 2020). Tafesse et al. analyzed 6 stress reactive characters phenotypically in 135 genotypically variant pea cultivars with controlled temperature stress. For this study, 16,877 recognized SNPs utilized to identify marker associated traits for stress tolerance. These novels markers and alleles impede way towards generation of temperature tolerant pea accessions (Tafesse et al. 2020).

3.7 Crop Wild Relatives

Crop wild relatives contain useful genetic variation. The categorization of *Pisum* depending upon physiology and karyology has varied over period (Kosterin and Bogdanova 2008) but remains problematic (Kosterin 2017). Research in topographical centers of origin integrating ecologically, morphologically, as well as genetically are required for better recognition and analysis of adaptation in crop wild relatives. Establishing hybrid inhabitants with crop wild relatives provide farmers a method for the detection of wild alleles (Coyne et al. 2020). *Pisum* having small genera with only about 2–3 notable classes (Kreplak et al. 2019); the *P. sativum* subsp. *sativum* and natural subspecies. *elatius* is innate to the Mediterranean territory of Europe and center region as well as north-west Asia, while the *Pisum fulvum* is specific to the Center-east region (Smýkal et al. 2017). *P. abyssinicum* was recognized in Ethiopia as well as in Yemen was possibly domesticated individually of *P. sativum* originated from subspecies *elatius.* The prime gene pool composite from *P. sativum* and sub species of pea (Trněný et al. 2018), but there are some barriers for its presence due to genetic movement (Bogdanova et al. 2009; Nováková et al. 2019). The secondary gene pool expands to another species, *P. fulvum* as well as *P. abyssinicum. P. abyssinicum* is not identified in CWRs however, in consist different assortment and karyotype (Trněný et al. 2018; Weeden 2018). The tertiary genetic pool of pea currently includes of *Vavilovia formosa* (Mikić et al. 2013b).

Hanci and Cebeci (2019) conducted a study to identify the connection among wild pea cultivars *Pisum fulvum* L., *Pisum abyssinicum* L., *Pisum sativum* var. *elatius*, localized cultivars *Pisum sativum* var. *sativum* L. and *Pisum sativum* var. *arvense* L. as well as some marketable cultivars ("Boogie- Rondo"). Diversity investigation carried out by using fourteen simple-sequence markers and fifty phenotypic characters. In total 48 alleles were identified during the molecular study. A total fifteen genotypes grouped into two leading clusters utilized for genetic examination for enhanced germplasm.

3.7.1 CWR and Their Geographical Distribution

Natural pea subsp. *elatius* with the extreme genomic variance around East region of Turkey, Syria, and Israel has a wide-ranging division with dispersed inhabitants over the Mediterranean as well as middle Asia, while *Pisum fulvum* division restricted to the center-east (Abbo and Ladizinsky 2015; Smýkal et al. 2017). The wild pea cultivars are genetically distinct due to geographical distribution but not due to climate which show a mixed mating approach with principal of self-pollination. Niche simulations with possible climate change cause decrease in ecosystems appropriate for wild peas, thus implicating convincing reason for further cultivation as well as ex situ preservation (Smýkal et al. 2018), While, Hradilova et al. recently stipulate scaffold for research on environmental effect on seed dormancy as well as growth patterns and seed coat configuration. The major difference among dormant/non dormant seeds was in viscosity of seed coat because of the quantity of proanthocyanins in it. Also, it is revealed that dormant cultivars are obtained in elevated temperatures and contain constricted temperature range (Hradilová et al. 2019). The legume plants with physical dormancy, seeds mainly grow in the autumn. As outcome, present seedlings take advantage of existing soil humidity and are ready for spring, thus circumventing elevated heat and water deficiency at flowering and seed filling. Temperature therefore is most noticeable ecological aspect for seed dormancy and growth. However, cold is not appropriate in several areas of wild peas for the beginning of flowering. Due to this, not all crop wild relatives associates of Mediterranean and central-east origin are long-day crops demanding convinced thresholds to start flowering in lentil as well as in pea (Weller et al. 2012).

3.7.2 Extent of Genetic Diversity in CWR

Awareness of current genomic variance in cultivated species and CWR is critical for genetic improvement, a typical source of tolerance to various biotic as well as abiotic stresses. The use of CWR in farming, however, leads to the emergence of unwanted wild type characteristics, transformed through the cycle of domestication (Meyer and Purugganan 2013). Improved genetic scans to identify CWR alleles conferring adaptation to abiotic stress are available and population genetic research can identify loci with elevated genetic proposition variance values, ultimately indicating loci with different selection for stress acclimatization (Coyne et al. 2020). New technology with latest software is also available with improved capability to discover SNP environment relatives. (e.g., Gradient forests, bayenv2, and Baypass, bayscan) (Fitzpatrick and Keller 2015). High throughput sequencing facilitates the identification of candidate genes and genetic mapping in environments that identify alleles adapted to different abiotic stresses allow selection of crop wild relative cultivars for further genome identification and insertion of those alleles into leading cultivars

(Sanderson et al. 2019). Wild pea provides sources of various abiotic stress tolerance, one of which is cold tolerance. The *Hr* flowering locus which is implicated in winter frost tolerance may be used to develop winter resistant pea cultivars (Lejeune-Hénaut et al. 2008). The water deficiency resistant characters crop wild relatives of pea cultivars comprises leaves greasiness, root architecture, and rhizobial relations. *P. fulvum*e reveals inferior drought vulnerability and may possibly be a basis for draught resistance (Mikić et al. 2013a). In addition, two pea subspecies *elatius* were identified with lower trypsin inhibition (TIA) inside seeds. High heritability helps to increase selection efficiency and genetic advantage in field pea cultivation (Kumar et al. 2019b).

3.8 Association Mapping Studies

3.8.1 Linkage Disequilibrium (LD)

In plants, the degree of LD and genetic diversity is the outcome of compound evolutionary history comprising choice of desirable alleles, domestication bottlenecks, and insertion of genetic material from CWRs into cultivars. These are of research in crops gained incredible attention for farmers and scientists (Vigouroux et al. 2002; Ross-Ibarra et al. 2007). The effect of global warming and highly increased population due man-made activities raised curiosity for the improved characterization of genotypic as well as morphological traits in plants. There is wide spectra of phenotypic dissimilarity in pea, because of diverse farming practices and also there is only few genetic areas have been characterized using QTL mapping (Siol et al. 2017).

LD based mapping deals high declaration since it is dedicated on utilization of SNPs and so has the capability to distinguish among closely associated cultivars (Brachi et al. 2011; Dhanapal et al. 2016; Jiang et al. 2017; Gali et al. 2019b). The advancement of improved genotyping methodology allows one to scan genome to categorize area of interest on basis of diversity, LD, allelic diversity, and additional genomic characteristics in crops. Association mapping intensively applied for characterization of many genomic loci as well as genes for important traits pea (Siol et al. 2010; Schmutz et al. 2014).

3.8.2 Target Gene-Based LD Studies

In *Pisum sativum* L., association mapping is utilized to identify many genome based traits comprising seed quality as well as agronomic traits (Cheng et al. 2015; Gali et al. 2019b), composition of lipid in seed and mineral concentration (Ahmad et al. 2015; Diapari et al. 2015), disease resistance (Sudheesh et al. 2015), as well as cold

and salinity tolerance (Leonforte et al. 2013b; Klein et al. 2014). A total of thirty-two marker trait associations were characterized by 16,877 known SNPs that were consistent in three environments with stress tolerance, six for chlorophyll absorption; two for photochemical reflection index (PRI); seven for generative stem measurement; six for internode distance; and nine for dimension of pods. A total of 48 genes were identified within a 15 kb region with the potential for marker-assisted selection to develop heat stress resistant cultivars (Tafesse et al. 2020). 92 pea accessions were assessed at 9 environments and genotyped using 1536 SNPs from which total 60 SNPs significantly associated with days to flowering, quantity of propagative nodes, duration for flowering, quantity of pods stem, percentage retention of pods (PRP), pods set precent (PSP) and decrease in pollen germination because of elevated temperature. Among those 60 MTAs, 33 SNPs were related to flowering initiation, 8 with pod growth, and 19 with reproductive node number. For genetic enhancement in pea, the regions of genes related with reproductive improvement stipulate incredible ground (Jiang et al. 2017).

3.8.3 Genome-Wide LD Studies

Genome wide association mapping is an effective method for identification of characters by natural genetic variance (Korte and Farlow 2013). It gives rise to greater mapping resolution compared to conventional bi-parental cultivars which efficiently helps to distinguish between molecular markers and characteristics (Liu et al. 2016; Cui et al. 2017; Mishra et al. 2017). This study needs characterization of population construction of the variety which supports to analyze genetic connection of individual and to eliminate false relatives (Korte and Farlow 2013; Sul et al. 2016). Recent advancement in high throughput Next generation sequencing platforms and single nucleotide polymorphism genotyping deliver supplementary resources for high-resolution characterization of genetic diversity and enable breeders to select useful diversity for the creation of new variants. In a recent study, 135 accessions were collected from various worldwide pea breeding agendas utilized for Genome wide association mapping. The accessions were genotyped with the help of genotyping by sequencing technology and assessed in multiple location, multiyear experiments for seed quality as well as agronomical traits (Gali et al. 2019b).

3.8.4 Potential of Association Studies for Genetic Enhancement

Association mapping, using a wider genetic cultivars, germplasm as well as breeding cultivars offers a powerful method to classify creative functional variants and quantitative character in crops (Rafalski 2010; Hamblin et al. 2011). Association mapping

has many benefits over conventional mapping: (1) using wider genetic diverse cultivars for MTA, (2) finding superior resolution mapping based on additional recombination for germplasm improvement, (3) accessibility of prior morphological data, and (4) can save time in production of bi-parental cultivars (Abdurakhmonov and Abdukarimov 2008; Hamblin et al. 2011). When assessing marker density, association mapping have potential to accelerate genomic studies (Siol et al. 2017). This is very essential for the crop like pea which are having huge genome. Though genetic variance as well as genome structure is characterized in many pea collections. The analysis of linkage disequilibrium patterns noticeably uncommon (Jing et al. 2007). The recent increase in marker accessibility throughout the genome (Tayeh et al. 2015b), it allows the assessment of genomic variance at the species level and development of new pea cultivars.

3.9 Molecular Mapping of Resistance and Quantitative Trait Loci

In Pea, early genetic mapping was done using morphological, physiological and isozymes as well as with genomic markers (Ellis et al. 1992). Markers are categorized in three major types, (1) physiological markers which have two sub type('classical' or 'visible') used to check phenotypes (2) bio-chemical is the genetic variant marker of isozymes and (3) DNA markers utilized to identify traits (Winter and Kahl 1995; Jones et al. 1997).

The genomic diversity estimation is carried out by various molecular techniques like RFLPs, RAPDs, AFLPs, and SSRs (Waugh et al. 1997; Karp et al. 1998) has been applied in many crops. In pea, genetic diversity analyzed using RAPDs, AFLPs and RFLPs and SSAPs (Bagheri et al. 1995; Samec and Našinec 1995, 1996). Amplified fragment length polymorphisms valuable to examine variance in pea cultivars due to its high level of polymorphism (Ahmad et al. 2015). Microsatellites utilized to evaluate 164 pea accessions for variability and structural analysis (Smýkal et al. 2008). AFLPs used to assess polygenetic relationship and variability for 21 pea cultivars in Germany (Simioniuc et al. 2002). Dyachenko et al. (2014) assessed difference among pea cultivars by AFLP markers. Recently, El-Esawi et al. (2018) used amplified fragment length polymorphism to evaluate connection of 25 pea cultivars and revealed correlation amongst the level of variance and salt resistance by MTA investigation in pea. SNPs are currently the genetic markers of choice as they are evenly distributed among the genome, are bi allelic and co-dominant. For SNP detection, numerous technology have been established from array based single nucleotide polymorphic genotyping system such as Infinium, Affymetrix and GoldenGate® depending upon quantity of samples has to be analyzed (Deulvot et al. 2010). For pea, the SNPs with ESTs were established to form a ample linkage map and 705 SNPs were successfully characterized. The SNP assay and genetics linkage

map association allowed to identify salinity tolerant QTLs and candidate gene in pea cultivars (Leonforte et al. 2013b).

3.9.1 Mapping Software Used

In genetic mapping approach, linkage evaluation is primary method in genomic dissection of QTLs in biparental segregation populations. To date various QTLs have been identified for quantitative traits, and some QTLs utilized to perform MAS in farming and to clone genes. Recently various software for QTL mapping have been published. Lander and Botstein (1989) carried out interval mapping with QTL/Mapmaker software. However, It does not show the influence of other quantitative trait loci on the genetic mapping of the studied QTL. To overcome this issue, composite interval mapping is suggested (Lander and Botstein 1989; Jansen 1993; Zeng 1994). For CIM, numerous software has been released. To advance this technology Genome-wide composite interval mapping is introduced for biparental segregated cultivars. For promoting this technology, R platform has been developed consisted by versions which is useful for dentification of QTLs in double haploid (DH) cultivars, RILs, back cross and F2 populations (Zhang et al. 2020).

3.9.2 Classical Mapping Efforts

In 1998, the pea genomic linkage map was constructed with RAPD markers to check its reproducibility in 139 RILs. A mapping population resulting from a cross between highly branched mutant named K586 derived from the pea accession 'Torsdag' and a dry seed accession cultivar 'Te' 're' 'se' was developed. The map currently consists of nine connecting groups covering 1139 cM. This map developed by RAPD consisting of 355 markers in 7 LGs. The main purpose of this map is to deliver a context for genetic studies, specifically for the localization of mutations, genes and QTLs regulating plant architecture (Laucou et al. 1998).

For pea the various techniques such as AFLP, RFLP as well as RAPD used to generate linkage map (Gilpin et al. 1997).

The linkage maps are initial steps for identification of qualitative and quantitative traits, introgression for necessary genes and QTLs and for positional cloning for economical important traits (Semagn et al. 2006). Many markers utilized for the creation of genetic linkage map for pea i.e., SSR, SNPs, ISSR and STS (Loridon et al. 2005; Deulvot et al. 2010). Guindon et al. (2016) used SRAP markers for the development of map in pea by using F_2 population resulting from a cross among DDR11 to Zav25 with 25 SARP primer combinations generating 208 polymorphic bands. The subsequent map has 112 genomic markers dispersed along seven LGs which covers total 528.8 cM. The lengths of the LGs range from 47.6 to 144.3 cM with 9-34markers (Fig. 3.5).

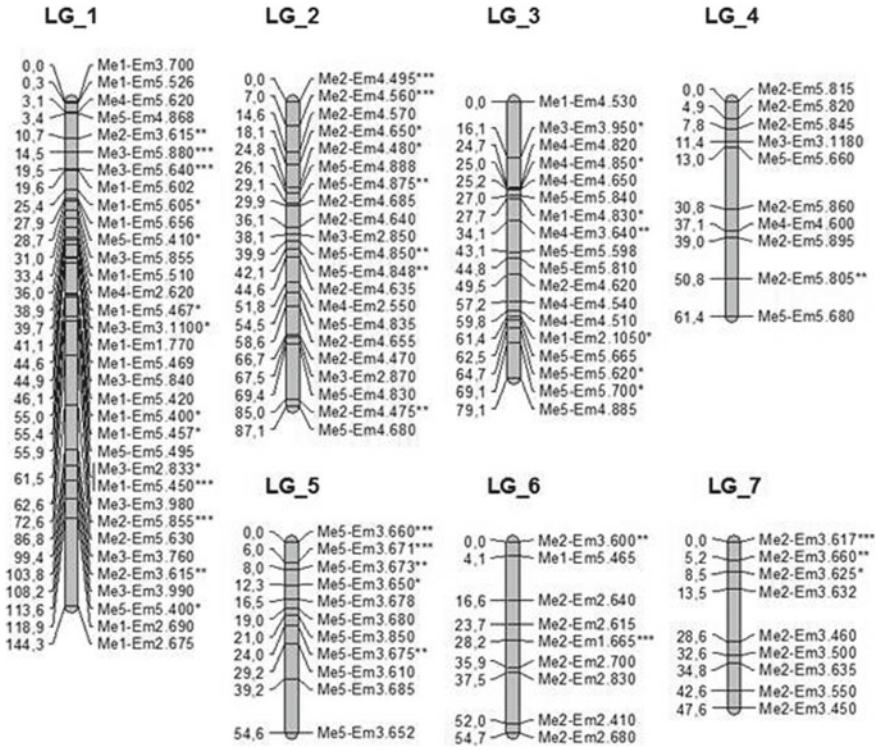

Fig. 3.5 Linkage map of *Pisum sativum* L. (Guindon et al. 2016)

3.9.3 QTL Mapping

Many QTLs were identified for various abiotic stresses which could be helpful to generate stress resistant pea cultivars (Iglesias-García et al. 2015). For example, formetribuzin tolerance, QTLs were identified on LG IV accounting for 12–21% of phenotypic variance (Javid et al. 2017). Based on multiple environmental factors, 375 quantitative trait loci are recognized for significant characteristics incorporating flowering days, maturity days, resistance to lodging, seed mass, grain yield fiber absorption, seed starch absorption, seed structure, and seed nutrient concentration (Gali et al. 2018).

3.9.3.1 QTL for Frost Tolerance

In pea, identification of frost tolerant genomic regions initially was carried out through analysis of mapping populations. For frost damage, mapping was carried out in various atmospheres and under control conditions. In the study, two RIL populations, Pop 9 (Champagne crossed with Terese) and Pop2 (China crossed with

Cameor), derived from cross among frost resistant and sensitive cultivars were used (Dumont et al. 2009; Klein et al. 2014). Within both populations, four common QTLs were located on LG3, LG5 and LG6. Recently, 62 SNPs were found by GWAS which correlated significantly with cold tolerance and are dispersed over six linkage groups as shown in Fig. 3.6, with biparental population. 50 candidate genes identified from annotated SNPs identified for frost stress related locus. LD study amongst pairs of SNPs were analyzed in span of 900 markers using software-Plink v.1.9 (Beji et al. 2020). The comparative genetic map of GWAS for QTL mapping for frost tolerance is described in Fig. 3.6.

GWAS study in pea has confirmed QTL for frost tolerance. QTLs considerably related with cold stress like winter frost damage WFD 3.2, 5.1 and 6. Also, CBF transcription factors function as possible genomic elements of the cold resistance locus on LG5 were highlighted (Beji et al. 2020). The delayed flowering gene in European winter pea *Hr*, with essential quantitative trait loci for cold resistance is also characterized. This indicates that genotypes of delayed flowering pea probably have a greater chance of being frost tolerant (Lejeune-Hénaut et al. 2008).

3.9.3.2 QTL for Drought Stress

Identification of genomic regions controlling drought stress has been carried out by assessment of RWC in soil as well as in leaf by providing water stress to pea. Inpea, 10 QTLs were identified for drought adaptation from RILs of a cross involving P665 and Messire. These QTLs linked with 9–33% phenotypic variant traits. In addition to this, the regenerative markers related to these quantitative trait loci were also recognized which can be utilized to select cultivar for drought adaptation in pea breeding programs (Iglesias-García et al. 2015). There are 3 QTLs correlated to root size are represented on Chr. 3 as well as 4 which were also related to fungal disease tolerance (Fondevilla et al. 2010, 2011; Ye et al. 2018).

3.9.3.3 QTL for Salinity Stress

For salinity tolerance, SNP markers associated with ESTs were established to create linkage maps in *Pisum sativum* L. pea. A total 36,188 different nucleotide positions were detected from which 768 have been designated for genotyping in a recombinant inbred line population (Kaspa × Parafield). Putative SNPs were identified using NextGENe software v1.96, and QTL discovery was carried out by MapManager QTX software. There was total 705 single nucleotide polymorphism (91.7%) identified by segregation. The quantitative trait loci for salinity resistance were recognized on linkage group 3 and 4 for identification of tolerant cultivars as shown in Fig. 3.7 (Leonforte et al. 2013b).

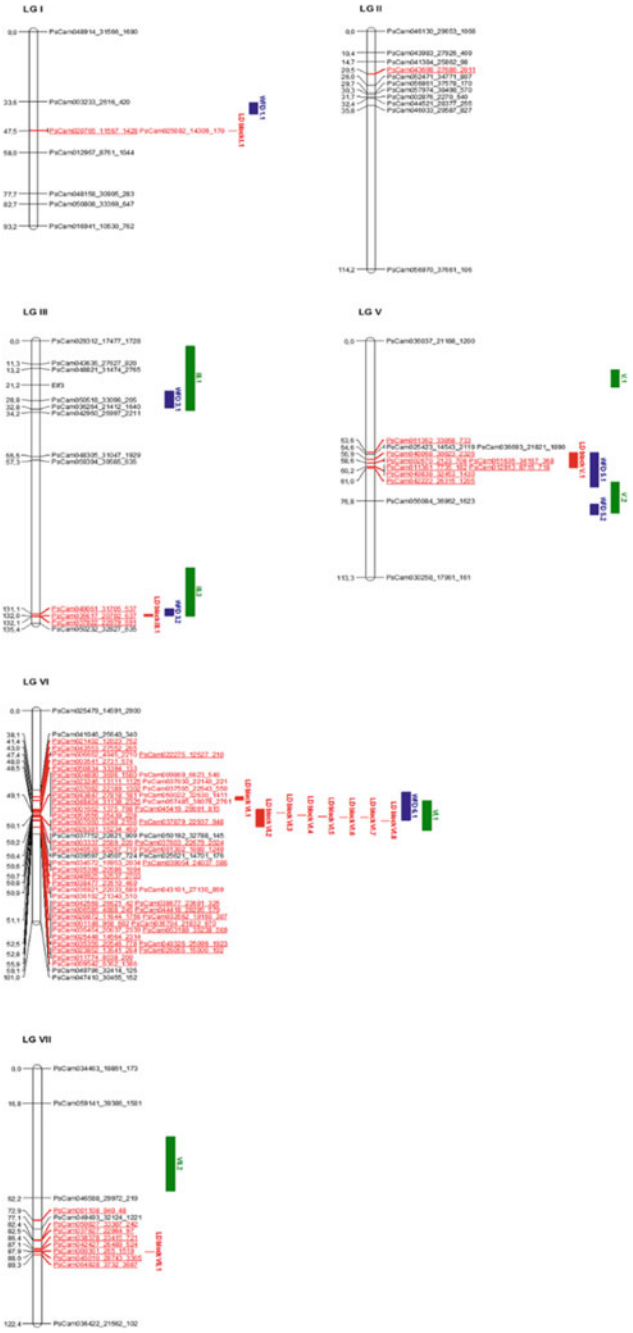

Fig. 3.6 Genome wide associated map loci compared with identified QTLs for cold tolerance in *Pisum sativum* L. (Beji et al. 2020)

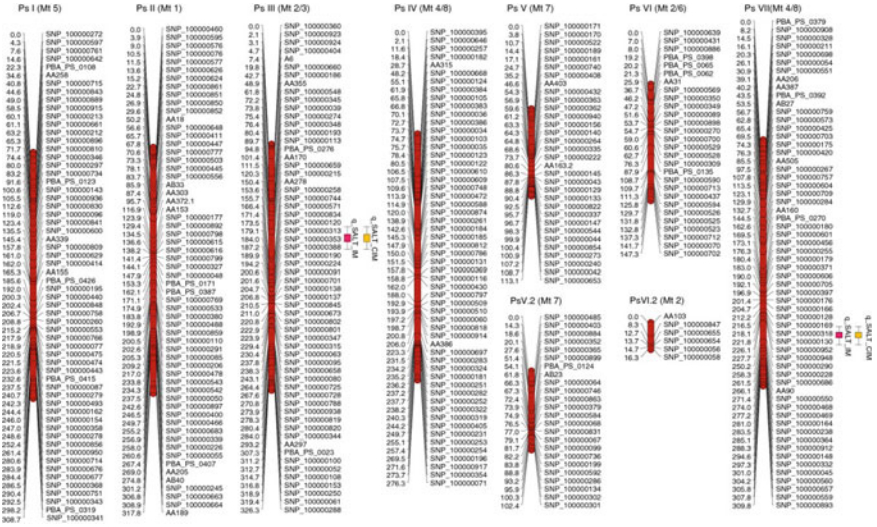

Fig. 3.7 Linkage map indicating salinity resistant QTLs (Leonforte et al. 2013b)

3.9.3.4 QTL for Lodging Resistance

Few studies have been carried out for this complex QTL as it is highly influenced by many external factors (Banniza et al. 2005; Tar'an et al. 2003). In recent studies, three RIL populations of pea i.e., PR 02, 07 and 15 containing of 94 cultivars had been phenotyped and genotyped for this trait that were utilized to map QTL. Among these mapping cultivars, lodging resistance quantitative trait loci were found on LG IIIb for PR 02, 07 populations while in PR15 it was identified on LG IIIa (Warkentin et al. 2004; Gali et al. 2018). GWAS in pea using a cross between 'Aragorn' (PI 648,006) and 'Kiflica' (PI 357,292) population, extra locus on chromosome 1, 2 and 3 were found for water lodging tolerance (Gali et al. 2019b).

3.9.3.5 QTLs for Heat Resistance

In pea, 107 RILs between CDC Centennial × Sage was used to map QTLs for days to flowering termination, pod number, 1000 seed weight and seed quantity/pod. A genomic linkage mapping was developed using SNP markers which consisted of 1024 loci with coverage of 1702 cM. Ten QTLs were identified consistent over environments with heat stress, and five each for flowering and yield traits (Huang et al. 2017).

3.9.4 Mendelization of QTLs

Gregor Johann Mendel in 1865 took pea as a model plant. Mendel's research was established by utilization of statistical analysis for qualitative characters. Till date 100 + crop genomes have been sequenced. Depending on Mendel's work, isolation of genes and their use in segregation of molecular markers have been developed. Genomic selection (GS) appears as a novel approach for the enhancement of genetic improvement in crop plants. Advanced phenotyping and development in statistical tools help in investigation of complex traits. Insertion of innovative genes from wild relatives as well as landraces broadens genetic variance for enhanced traits; transgenic technology helps for introgression of new genes (Smýkal et al. 2016). Castle (1903) explained the applicability of Mendel's laws by giving the examples for pea intermediate height from short X tall cross. The name quantitative trait locus (QTL) was coined in 1975 (Geldermann 1975). For mapping, new techniques were proposed including association mapping with pools of genotypes which capture important meiotic stages (Risch and Merikangas 1996). The arrival of whole genome sequencing has allowed the "mendelizing" of QTLs, where there is no limitation of genomic markers (Hori et al. 2016). Mendel's work created the base for recent genetic engineering and molecular farming techniques with the addition of GWAS and GS for improved pea breeding lines (Smýkal et al. 2020). Genomic selection (GS) is used to improve grain yield of pea under severe drought in the Mediterranean environments using simple nucleotide polymorphic markers from genotyping by sequencing and also comparison done for Genomic selection with morphological selection as well as marker-assisted selection. In detrimental draught condition, the genomic selection revealed 18% higher efficacy compared to marker assisted selection and proved to be economical (Annicchiarico et al. 2020).

3.10 Marker Assisted Breeding (MAB) for Resistance Traits

This technique helps to identify genes prior to trait been expressed in crops as it can identify heterozygosity carrying recessive genes which do not show any trait (Warkentin 2015). An overview of MAB for germplasm is represented in Fig. 3.8.

3.10.1 Marker-Assisted Gene Introgression

Molecular breeding approaches to insert several traits include marker assisted back-crossing (MABC), marker assisted gene pyramiding, marker assisted selection (MAS), marker assisted recurrent selection (MARS), genomic selection (GS), as well as genome wide selection (GWS) (Rana et al. 2019). MAS is an efficient method

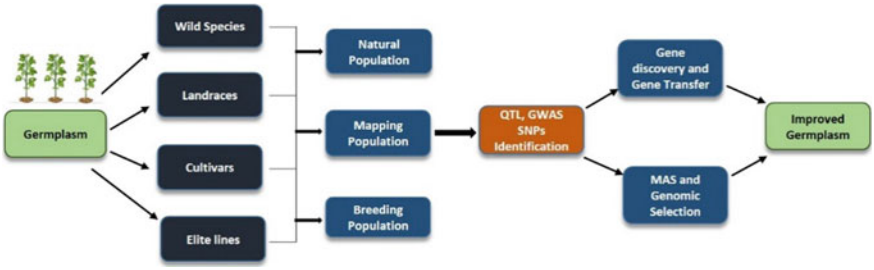

Fig. 3.8 An overview of marker assisted breeding (MAB)

for analyzing thousands of genome regions for improving economically essential traits under conditions of water and salt stress. RFLPs, APLPs, RAPDs, SSRs, and ISSRs are used for various plant genetic analyses. These markers are not produced from the genes because gene cloning in polyploidy crops with big genome sizes is complicated. On the other hand, functional markers from within transcribed regions of functional genes are associated in a significant way with gene function and may be involved directly in targeted selection. Wild plants contain genetic variation and novel alleles. Wild plants are therefore considered a possible source of gene(s) for developing resistance to biotic as well as abiotic stress. The use of functional markers and genomic transformation are valuable technique for desired insertion of genes to generate tolerant cultivars. To promote the transition of abiotic stress resistant cultivated plants, molecular breeding strategies are employed. The use of molecular markers effectively facilitates gene crossing from wild species to inbred lines or elite cultivars (Ahmad et al. 2020).

There are various benefits of MAS over traditional quantitative trait loci mapping (i) Use of broader genetic variants, (ii) to obtain higher resolution mapping population, (iii) Bi-parental population generation is not required, (iv) utilization of developed phenotyping data (Abdurakhmonov and Abdukarimov 2008; Hamblin et al. 2011). MAS has been effectively implemented in various plants like soybean, maize, and also in pea for important agronomic traits (Kwon et al. 2012; Cheng et al. 2015; Diapari et al. 2015). MAS analysis for cold resistance in pea executed with 267 simple sequence repeat markers, from which 16 winter hardy cultivars were identified. Seven associated markers were detected which are associated with frost tolerance out of which one functional marker on linkage group 4 with response to chilling stress in pea was identified (Liu et al. 2017). Recently, MTA analyses have been reported in pea for heat tolerance using 16,877 SNPs from which 32 MTAs were found to be consistent over 3 different environment condition in which chlorophyll concentration—6, reproductive stem length—7, pod number—7 and internode length—6 were identified. These markers have capacity for MAS for improvement of temperature tolerant pea-cultivars (Tafesse et al. 2020). Marker-assisted backcrossing (MABC) strategy was similarly applied for introgression and 3 frost resistant QTL were recognized in pea (Lejeune-Hénaut et al. 2008). Apart from tolerance to various abiotic as well as biotic stress MAS was also used to develop pea cultivars on the basis of folate profile

from eighty five accessions by GWAS (genome wide association study), which led to identify SNP markers related to folate profile of pea (Jha et al. 2020).

3.10.2 Gene Pyramiding (GP)

It is an important technique of grouping different alleles from various donor parents into a single genotype. While it is possible to pyramid many genes through conventional methods of plant breeding, it is difficult to phenotypically select and identify a single plant that contains more than one favorable gene for different traits. There are chances of loss of genes of interest from recombination (Iyer-Pascuzzi and McCouch 2007). Marker assisted selection can improve the prospect of gene pyramiding for different traits (Chukwu et al. 2019). GP of overexpressed EaDREB2 with the pea DNA-helicase gene has been reported which enhanced water deficiency and resistance to saline soil in sugarcane (Augustine et al. 2014).

3.10.3 Limitations of Marker Assisted Selection

Botstein et al. had established first DNA markers in 1980s (Botstein et al. 1980), followed by Beckmann and Soller (Soller 1986) who named as marker-assisted selection (MAS). It took another ten years before (Concibido et al. 1996) published the first comprehensive paper to apply MAS in crop farming. Accuracy of QTL estimation is necessary for successful MAS and is mainly affected by the level of replication utilized to generate phenotypic data and population size used for mapping. So MAS is reliant on accurate QTL mapping studies (Brumlop and Finckh 2011). For successful application, MAS requires an optimized strategy and integration with phenotypic selection (Lema 2018).

3.11 Map-Based Cloning of Resistance/Tolerance Genes/QTLs

Many agricultural characteristics reflect composite quantifiable inheritance. To study these characteristics, the QTLs recognition supplemented with mapping as well as cloning is essential. Genomic technology advancements have modernized our knowledge of complex traits as well as trait-associated genomic regions have been employed in marker-assisted QTLs/genes cloning (Jaganathan et al. 2020).

3.11.1 Traits and Genes

Heat shock factors (HSFs) modulates heat stress in plants. Incorporation of heat shock factors from heterologous species into pea, such as the HsfA1d from *Arabidopsis thaliana* improves heat stress tolerance (Shah et al. 2020). Mitogen activated protein kinase were described as important factor for biotic, abiotic stress and for growth modulation in plants. The C1 subgroup MAPK from pea, PsMPK2 is induced in vegetative as well in reproductive parts in *A. thaliana* in response to stress signals such as abscisic acid, jasmonic acid and hydrogen peroxide. The C1 subgroup MAPKs of pea plays significant role in stress reaction (Ortiz-Masia et al. 2008). Studies on regulation of calcium sensor calcineurin B-like protein CBL interacting protein kinase have been carried out in pea by cloning of *PsCIPK* and *PsCBL* genes which play significant part signaling (Mahajan et al. 2006).

3.11.2 Genomic Libraries

Genomic libraries with large inserts are important resources cloning, mapping and sequencing for genome (Tanksley et al. 1995; Zhang et al. 1996). Many bacterial artificial chromosome (BAC) clones prepared for the major plants like soybean, rice, chickpea, pea as well as cowpea. These libraries were a major source for the identification of molecular markers for important agronomic traits. The BACend sequences derived simple sequence repeat markers are low cost (Temnykh et al. 2001) which also gives coverage for whole genome. Pea genome shares conserved synteny with related legumes. Pea BAC libraries were developed which is useful for the identification of alleles for crop disease tolerance of agronomical important traits (Coyne et al. 2007). There are 2 approaches of probing gene of interest are high density filters (Woo et al. 1994) and PCR amplified of BAC-clones from isolated pools (Green and Olson 1990). BAC pools allow facile interrogation of the complete BAC-clone library for Resistance gene analogues (Timmerman-Vaughan et al. 2000).

In pea, whole genome profiling—WGP is physical map based on sequencing tool, which utilize tags produced from NGS for the creation of BAC-contigs for genomes. This methodology of mapping offers outline for sequence as well as knowledge for identification of alleles which are challenging to search by positional-cloning. Also sequence-based physical maps derived from assembled BACs aided the assembly of the draft pea genome (Gali et al. 2019a).

3.11.3 Test for Expression

Transgenic technology allows the introgression of alleles from plant which are cross incompatible for precision crop improvement. DNA-helicases as well as RNA-helicases demonstrated their efficiency in many plants by enhancing resistance to salinity and water deficiency stress. capability of a Pea DNA Helicase to combat various abiotic stresses has been demonstrated in chili via *Agrobacterium* transformation approach (Shivakumara et al. 2017). The two groups of delayed flowering mutation for pea were isolated that describe two unknown loci, LATE BLOOMER-3 and 4, which have diverse effect on reproductive and vegetative development. Using a map-based clone technique, it was revealed that LATE 3, 4 are orthologs CDK8 as well as cyclin C1 which is intensively conserved in eukaryotic species. The genomic and physiological association of LATE3 and 4 shows contribution for genetic guideline of alleles responsible for the flowering (Hasan et al. 2020).

3.12 Genomics Assisted Breeding

3.12.1 Genetic Resources

Trait specific genes have been characterized for pea which are used as a valuable source for markers. Genetic maps for pea were developed and their homology recognized in species like *M. truncatula*, lotus, soybeans as well as poplars (Aubert et al. 2006). Bordat et al. developed a bioinformatics platform to identify sequence position on consensus map and to find candidate gene among neighboring unigenes (Bordat et al. 2011). Transcriptome sequencing provides valuable information about gene expression data. RNASeq. Information of juvenile pea nodules as well as root-tip were generated, and de novo assembly was made. This information possibly used as a genetic markers development, polymorphic study, and real time polymerase chain reaction (Zhukov et al. 2015).

3.12.2 Genome Sequencing

The inbred accession of *Pisum sativum* L. 'Cameor', which was developed by Seminor in year 1973, was sequenced draft assembly of its seven chromosomes published (Kreplak et al. 2019). Illumina short-read gene sequence (281X span of genome) were gathered to form contigs by Soapde-Novo2 and merged to form scaffold by PacBio RSII sequence (13X span of genome) and whole-genome profiling of BAC library (Kreplak et al. 2019). The size of pea genome assembly is 3.92 gigabytes which is ~88% of the assessed pea genomic size of ~4.45 Gb. Seven assembled pseudomolecules cover 3.23 Gb (82.5%) and 14,266 scaffolds covering 685 Mb

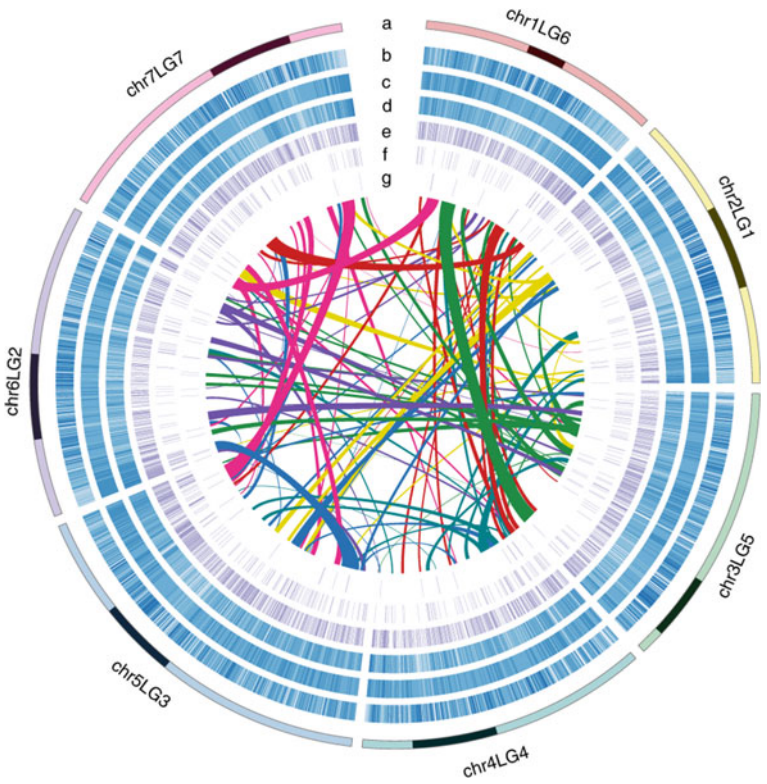

Fig. 3.9 Pea genome structure (Kreplak et al. 2019)

were unassigned. The gap in the estimated size is due to highly repeated sequences. The centromeric position is displayed by comparison of Skim GBS mapping with pseudo-molecules as shown in Fig. 3.9 (Kreplak et al. 2019). The genome sequencing of pea is expected to revolutionize the pea breeding (Tayeh et al. 2020).

3.12.3 Gene Annotation

Gene annotation is the process of defining structure (Exon prediction) or function of the gene. Availability of pea draft genome open up the scope of identification of structure and function of new genes related to different abiotic stresses which will help in developing novel stress tolerant varieties. In pea genome annotation method, 44,756 full genes and 29 truncated genes were identified. The average gene length is 2,784 base pairs (bp). The average coding sequence length is 1,016 bp. The average exon numbers are 6.33 exons (Kreplak et al. 2019).

3.12.4 Genomics Assisted Breeding Applications

High throughput sequencing technologies and availability of transcriptome data has enhanced gene expression studies and functional annotation of different abiotic stress related genes. RNA sequencing of cold-treated and control samples of two pea varieties Champagne (Ch) and Térèse (Te) was done. Differential expression analysis identified 1403 genes related to the chilling response and 1091 genes related to freezing tolerance (Bahrman et al. 2019).

3.13 Recent Concepts and Strategies

3.13.1 Targeting Induced Local Lesions in Genomes (TILLING)

It is a method to classify induced mutations in target alleles that which may be utilized for breeding. The mutagenized population is usually created either by using the ethylmethane sulphonate (EMS) which is potential chemical mutagen or fast neutrons and gamma rays which generate deletions of varying sizes (Till et al. 2003). TILLING is a very useful tool in model plants including legume model crop, *Medicago truncatula* and has become an important technique for reverse genetics. Over the last 10 years this process has changed with recent advancement of NGS permitting multiplexing of allelic targets in genomes. Thus, to accelerate workflow of TILLING, researchers have described the application of high throughput NGS technique (Tsai et al. 2011). TILLING has the benefit of being a non-GM (Genetically modified) methodology and therefore suitable in many plants. TILLING detects mutations in mutagenized populations while Eco-TILLING identifies single nucleotide polymorphism in wild cultivars and develop interest for farming to create novel traits. This is reverse genetic tool which can be utilized in various species regardless its size of genome and level of ploidy. Tilling by sequencing is preferred because of various reasons such as (i) The three dimensional pooling approach helps identify individual mutant without any extra sequencing steps, (ii) it allows to identify mutation in single nucleotide and its effect on precise trait, and (iii) it is not dependent on fluorescent primers (Irshad et al. 2020). The schematic workflow of TILLING with recent advancement is represented in Fig. 3.10.

In pea, research has been done to produce non-GMO cultivars which no longer express beta amyrin synthase by screening a mutant population created by EMS mutagenesis. The database UTILLdb has been developed which comprises phenotype and genotype information of mutant genes (Dalmais et al. 2008). One stream of pea research over the past decade is associated with increasing the concentration and bioavailability of important micronutrients. For this, the main effort has been to develop and evaluate low phytate pea lines as it is not well digested by humans or mono gastric animals. The pea lines identified having low phytate have

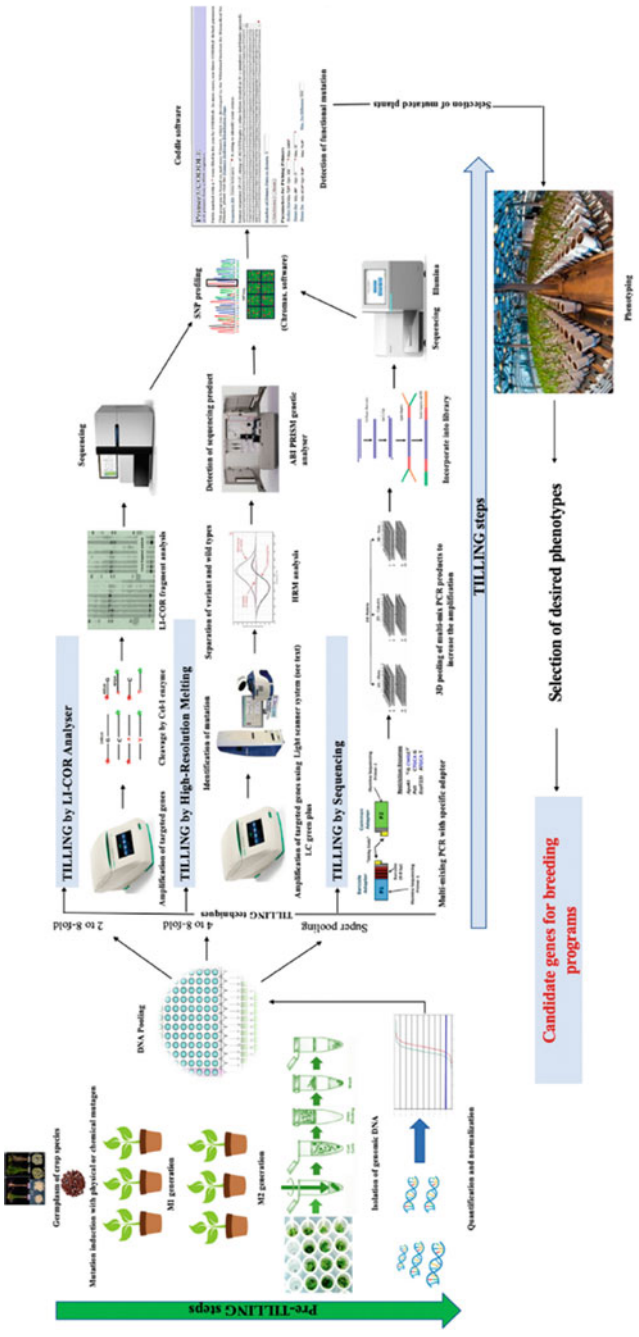

Fig. 3.10 TILLING workflow (Irshad et al. 2020)

the majority of their phosphorus in a bioavailable form and also deliver 2-threefold more iron to human cells. In addition to high protein, other important aspects for farmers include grain yield, lodging resistance, and physical seed quality. For this, germplasm resources and mutagenized populations of pea have been screened to identify null variants for genes encoding several seed proteins. Mutations created by fast-neutron mutagenesis in JI 2822 include large genomic deletions, of which have additional effect on two additional unrelated genes (Claire Domoney et al. 2013; Moreau et al. 2018). The accessibility of draft pea sequence for JI 2822 has helped to estimate minimum sequence deletion (Rubiales and Mcphee 2020). Chemical mutagenesis is not only used to understand role of specific amino acids but also used to develop various biotic and abiotic stress responsive genes to develop breeding lines. Two abiotic stress responsive competitor genes (DHN and CAD) show one sharp band for both genes under salinity stress in an EMS mutagenized population. These genes were previously confirmed for their significance in stress response in various other legumes (Hameed 2018).

3.13.2 Gene Editing

In recent years, CRISPR/Cas based gene editing systems, including precision base editing (Zong et al. 2017), has opened new possibilities in crop breeding. In combination with speed breeding technology, this method can be used to shorten duration of creating transgenic seeds with homozygous genotype (Ahmar et al. 2020). The regulation of genome-edited plants are rapidly changing in many countries to respond to emerging technologies. India recently called for public feedback to inform its decision-making on potential genome editing policies and published a draft paper on genome-edited species in January 2020: "Regulatory structure and risk assessment guidance." The current status of genome editing legalization in various countries is shown in Fig. 3.11 (Schmidt et al. 2020).

For pulses, the utilization of adaptive traits to be climate-smart can be achieved by using methods such as transgenics, genome editing, and epigenetics (Kumar et al. 2019a). The CRISPR-Cas9 is new and very effective genome engineering method in various plant species. The genome editing technology by CRISPR/Cas9 suggested that modifying RSA linked genes to enhance the tolerance of soybean under drought conditions (Sun et al. 2015; Jacob et al. 2016). However, the introgression of single guide RNA remains challenging. Crop researcher utilized TRV for VIGS proved efficient technique for facilitating functional genomics in various plant organisms. TRV genome made up of two + ssRNAs in which second RNA constructed for gene transmission via VIGS (Senthil-Kumar and Mysore 2014). These technologies may be used for selective mutagenesis, gene knockout generation and precise integration of regulatory or gene fusion sequences.

Recently, a detailed reproducible CRISPR-Cas9 framework for stably edited aphid lineages has been reported in pea (Le Trionnaire et al. 2019). To mitigate abiotic stress, the pea DNA- Helicase 45 via *Agrobacterium*-mediated transformation used

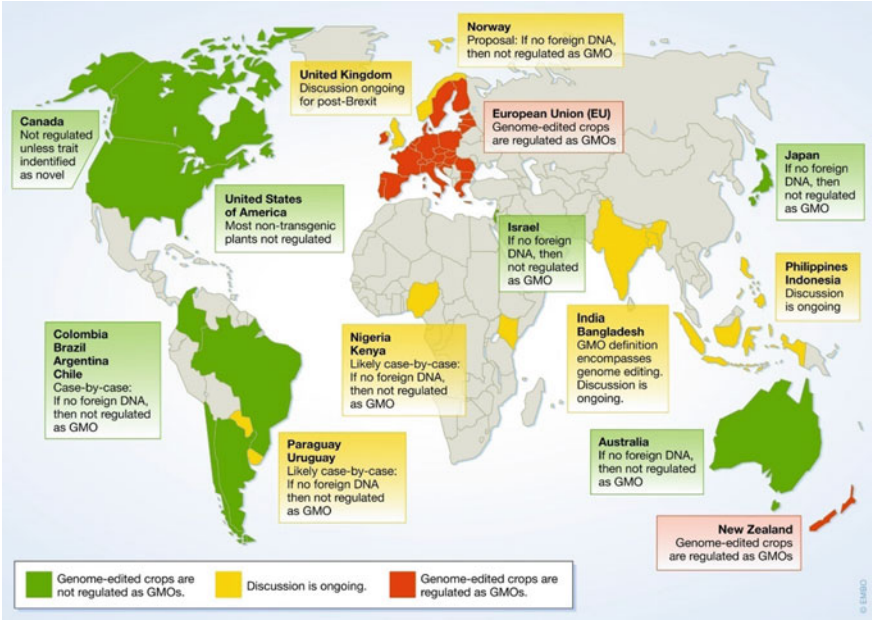

Fig. 3.11 Current state of genome-editing legislation (Schmidt et al. 2020)

which demonstrates the potential in combating multiple abiotic stresses. Transgenic events showed revitalized development and production with associated enhancement in WUE (Shivakumara et al. 2017). Ali et al. (2015) reported the genetically stable transgenic pea carrying salt tolerant allele (AtNHX1) transfected by *A. thaliana* over a period of six years with respect to morphological features such as leaf size, color and shape, tendril number, plant height, flower-shape, pod-shape, and grains. Compared with wild type, transgenic pea plants showed salt tolerance as well as frost tolerance. The transgenic pea plants also shows improved salt tolerance responses harboring overexpression of Na^+/H^+ gene from *A. thaliana* with association of PGPR (Ali et al. 2015, 2018).

3.13.3 Nanotechnology

Over the last decade, a number of patents have been granted in agriculture which have used nanomaterials in practice, e.g., nanofertilizers, nanopesticides, or nanosensors (Daniela Predoi et al. 2020). In the period of climate change, nanotechnology improves agricultural production by using nano-tools such as nano-biosensors, which support the efficient agricultural farm. The integration of nanotechnology and biology into nano-sensors has potentially increased their potential to identify and sense the environmental conditions or impairments (Shang et al. 2019). The use of

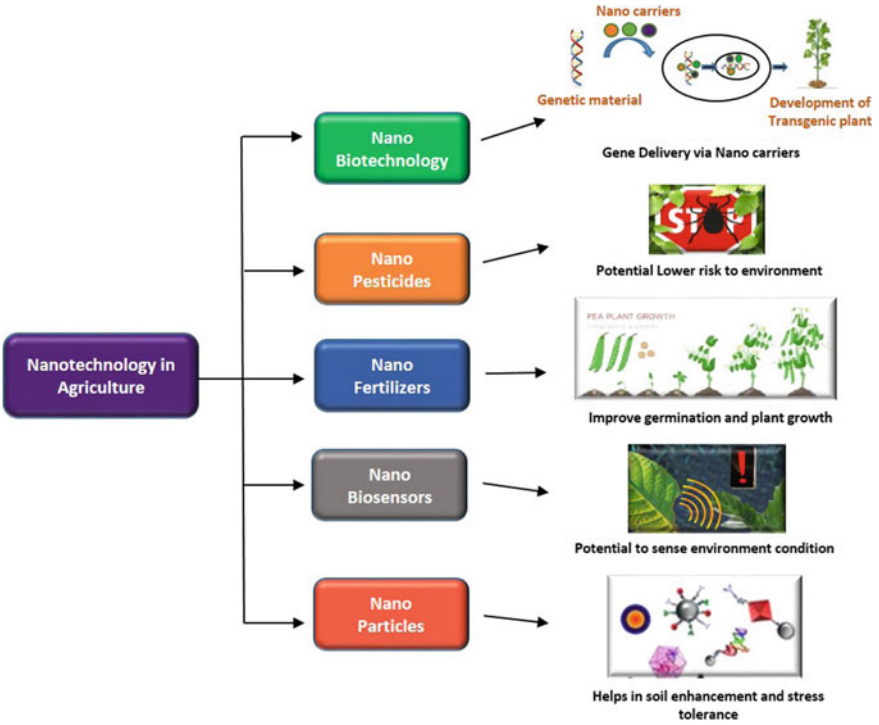

Fig. 3.12 Applications of nanotechnology in agriculture

nanotechnology offers promising applications for precision agriculture is described in Fig. 3.12.

Plant proteins have shown advantages over animal proteins in various food applications as an alternative "green" material (Nesterenko et al. 2013). Plant proteins shows less allergy reactions than animal due to its amphiphilic nature as well as its emulsifying property. (Jenkins et al. 2007; Li et al. 2012). Recently, nano protein particles had great interest due to its unique character and pea protein nanoparticles (PPN) have been isolated to identify variation in its structure, stability as well as function (Doan and Ghosh 2019).

3.14 Genetic Engineering for Resistance/Tolerance Traits

Genetic transformation is a valuable enhancement to the traditional breeding for abiotic as well as biotic stresses in pea (Warkentin 2015). The first report of a GM pulse crop, *Vigna aconitifolia* L. was in the 1980's but advancement in legumes are not as remarkable as cereals (Eapen et al. 1987; Kohler et al. 1987; Eapen 2008). The inadequacy of effective reproducible genetic transformation methods (Popelka et al.

2004) and cost of regulatory process are a major lag in GM pulse crop development (Kalaitzandonakes et al. 2007). Sustainable improvement of pea grain yield is always valuable for farmers along with resistance to various stresses like drought as well as heat at time of flowering, as well as frost and salinity stress tolerance. Other target traits include improving pea communications with beneficial species like mycorrhiza and rhizobia as well as adaptation to environmental stress, adjusting plant phenology and morphology to novel crop systems (Duc et al. 2015; Tayeh et al. 2015b; Foyer et al. 2016).

3.14.1 Gene Transformation

DREB Transcription elements are main regulators for environmental gene expression and stress responses. Transcription factors are ideal targets to compensate for the mutagenicity in crops, as they appear to target multiple pathways and participate in regulatory element manipulation (Hussain et al. 2011). During osmotic stress many genes are induced having conserved DRE (drought responsive elements), which are plant-specific and which associate with dehydration sensitive element DREB1 and DREB2 and their products may activate other genes involved water deficiency stress (Liu et al. 1998; Yamaguchi-Shinozaki and Shinozaki 2006).

Gene introgression via *Agrobacterium* methods in pulse crops to enhance economic importance is a valuable tool. However, some pulses are easier to transform than others (Dita et al. 2006; Eapen 2008). The main focus has been on herbicide resistance and insect resistance, but to date, farmers have not registered GM peas (ISAAA 2016). Creation of genetically modified insect-resistant pea (Zhang et al. 2015), improved source sink partitioning in transgenic pea, and drought tolerant pea have been reported (Kahlon 2019) but no field trials which show efficiency of said traits have been reported. However, in the oil seed legume, transgenic soybean with glyphosate resistant is one of the most successful genetic modification in Fabaceae family (Arruda et al. 2013). The new VrDREB2A gene, a DREB-binding mung bean transcription factor, enhance resistance for water deficiency as well as for salt in transgenic *A. thaliana* by activating downstream genes without growth retardation. It demonstrates that VrDREB2A is an essential transcriptional activator that can help increase resistance to abiotic stresses (Chen et al. 2016). In soybean, anovel DREB2 gene was identified which was majorly expressed under draught condition as well as in cold condition (Mizoi et al. 2013). Co-expression of transcription factor and PR protein in plants may have an additive effect in providing tolerance to drought. Very few GM crops have been approved with drought tolerance characteristics. According to ISAAA (2017), only two, Verdeca HB4 soybean and Genuity® Drought GardTM maize have been approved to date. Transgenic approaches to drought-tolerant crops are making considerable headway but consumer acceptance is still a challenge (ISAAA 2017). One current focus is also on translating findings from laboratory models into field crops (Deikman et al. 2012). In model legume plant *Medicago truncatula*, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE

8 (*SPL8*) gene was identified and down regulation of this gene in transgenic alfalfa showed significant enhancement in salt and drought tolerance by regulating various anthocyanin genes such as *CHS*, *PAP*1 and *DFR* which are intently related with abiotic stresses plants (Gou et al. 2018). Similarly, a transgenic heat tolerant line has been developed by incorporating heat shock factor, *HsfA1d*, isolated from *A. thaliana* by using pGWB415 expression vector (Shah et al. 2020). Transformed pea plants generated showed a fivefold increase in the expression of *HsfA1d* under heat stress (42 °C) by decreasing hydrogen peroxide and improving the activity of antioxidant enzymes compared to wild type plants.

3.14.2 Organelle Transformation

In modern years, genetic engineering in crops has been improved for crop improvement. Three plant cells carry genetic information such as nucleus, mitochondria as well as plastids (Bhattacharya 2010). The usual process to transform crops is the introgression of outer genetic material in nucleus by biolistic or *Agrobacterium* transformation. Plastid genetic engineering proposes another method for plant transformation with emerging approach such as nano-tubules made with carbon molecules for the gene introgression. Chloroplast harbor many copies of genetic material, transgenes introduced into the chloroplast genome may often achieve higher levels of protein accumulation so they are having major interest for novel agriculture approaches (Yu et al. 2020). This method helps to transform species which are transformation recalcitrant. Plastid genetic introgression is achieved successfully done in several plant species including legumes (Day and Goldschmidt-Clermont 2011). Plastid transformation methodology for pea was established by electroporation method for the induction of ATP citrate lyase (ACL) (Rangasamy et al. 1997).

3.14.3 Gene Stacking

The term gene stacking is used in agricultural research to designate breeding and GM techniques that target multiple traits at the same time. It could be the way of introgression of multiple genes in plants to confer tolerance to a single or multiple stresses (Taverniers et al. 2008). This could be achieved by introgression of many genes of the desirable trait/s simultaneously or by retransformation or by conventional crossing of GM plants or a combination of both (Halpin 2005; Taverniers et al. 2008). Many genetically modified crops with stacked traits are commercialized and in use by farmers (ISAAA 2017); reviewed by (Taverniers et al. 2008). But the limitations such as (i) unassociated stacked genes can get inserted at distinctive locus into genome which can segregate in subsequent generations, (ii) requirement of multiple selectable markers for individual gene co-transformed, and (iii) regulatory approval is needed (Halpin 2005). With advancement in multigene delivery systems, this process will

become more common in the future. For water deficiency and salt resistance tolerance in sugarcane, the induction of EaDREB2 and gene stacking of pea DNA-helicase gene with EaDREB2 achieved successfully (Augustine et al. 2014).

3.14.4 Gene Silencing

Virus induced gene silencing (VIGS) is reported successfully utilized to analyze function of genes into crops. However, it is not broadly employed in legume plants due to limitation with virus inoculation efficiency. With aggregated genomic data availability for legumes especially pea, it is essential to develop reliable approach for characterization of gene functions which helps to improve abiotic stress tolerance as well as for production, tolerance to pest and plant diseases. There are several advantages of VIGS such as (i) it can reduce the expression of more than one gene compared to lengthy plant tissue culture methods for producing transgenic plants, (ii) it shows capability to produce annotation linked to innovative traits in a one generation, (iii) the silencing of a series of genes without including conserved sequence of a gene into virus, and at last (iv) it allows to produce plants without embryo lethality. There are many viral-vectors applied to silence genes into pea plant (Burch-Smith et al. 2004; Hettenhausen et al. 2014). PEBV Pea early browning virus transfection successfully employed in pea which is hosted by *Agrobacterium*-mediated infiltration (Constantin et al. 2004). VIGS in pea plant also successfully reported in roots and leaves with single step method by bean pod mottle virus (Chouaïb Meziadi et al. 2017). The use of apple latent spherical virus is established in pea via *Agrobacterium tumefaciens* carrying a phytoene desaturase gene from *Phaseolus vulgaris* which has shown extremely uniform knockdown phenotypes (Xiong et al. 2019).

3.14.5 Prospects of Cisgenics

The first GM plants was denoted by Nielson 2003 in various categories based on phylogenetic distance between DNA donor and recipient organism and suggested that evolution of GM plants should be considered according to these categories. Schouten, Krens and Jacobsen introduced cisgenics concept in 2006 with high anticipation of cisgenics crops which can be more acceptable to the consumers (Schouten et al. 2006). Cisgenics is a gene derived from a gene pool of sexually compatible species which is naturally existed and it is an identical copy of endogenous gene in sense orientation, including flanking regions, introns, promoters and terminators. For cisgenesis, if transformation is based on Agrobacterium method, in vitro rearrangements are not permitted, T-DNA border sequence also can be introduced (Holme et al. 2013). The crucial difference between cisgenesis and transgenesis is the source of the gene. Cisgenic organisms are totally different from transgenic ones (Schouten et al. 2006). In transgenic approach control sequences are exogenous

usually originate from species that is neither recipient nor a close, sexually compatible relative. Transgenes are traditional type of isolated gene available for GM approach (Jacobsen and Schouten 2009). The new development for novel biotechnological approach is required for improvement of quality and quantity of plant crops. The most efficient strategy of transgenesis is having a political, ethical, and social fears so now a days new plant breeding technique, called 'cisgenesis' is intensively studied and claimed that cisgenesis is an alternative to transgenesis for safe and eco-friendly agriculture (Dudziak et al. 2019). Schematic representation of different types of genetic transformation for the development for climate resilient pea is described in Fig. 3.13.

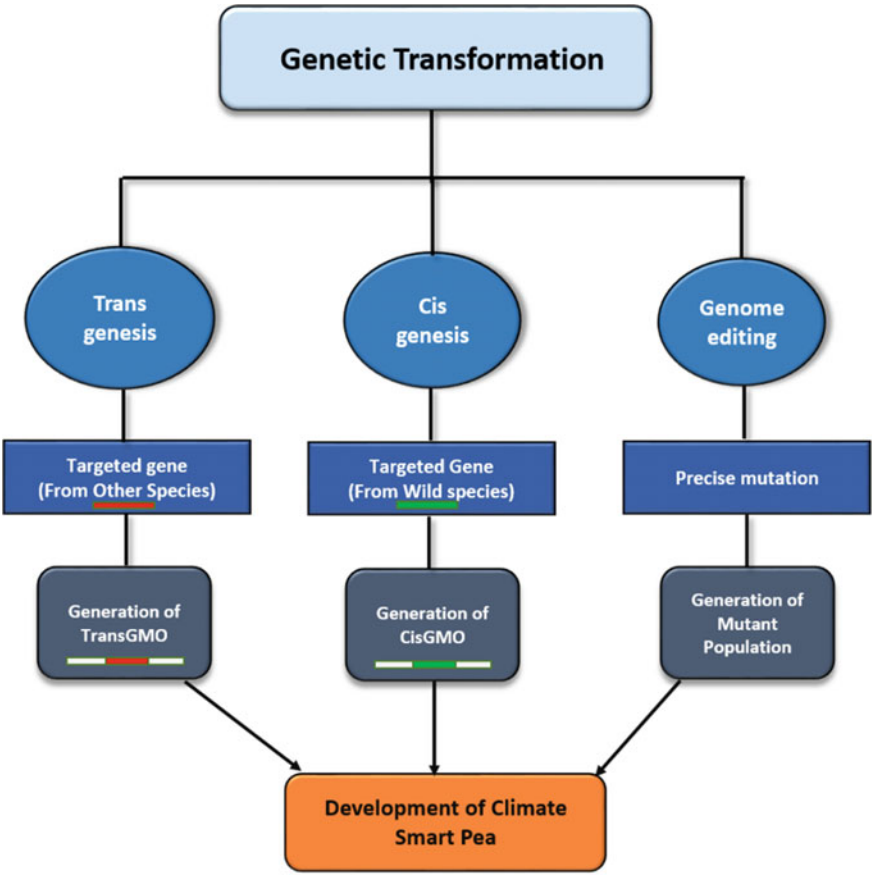

Fig. 3.13 Approach for development to climate smart pea by genetic transformation

3.15 Bioinformatics Tools

3.15.1 Gene and Genome Database

Research on generating genomic resource for pea and identifying genes involved in response of different stresses are very helpful to develop pea varieties for resistance to abiotic stresses like cold, salt, and drought. Apart from the recently sequenced pea genome (Kreplak et al. 2019), several genomic resources have been generated for pea including the atlas of pea genome (Alves-Carvalho et al. 2015), Databases developed to access genome data includes NCBI (National Center for Biotechnology Information), The Legume Information System (LIS) (Dash et al. 2016), URGI (INRA) and Pulse Crop Database (PCD).

3.15.2 Gene Expression Database

As the pea genome is large and complex (Macas et al. 2007), transcriptome sequencing can be used to provide information on gene expression (Wang et al. 2009; Ozsolak and Milos 2011). The high-quality pea transcriptome data was generated, and raw reads were included in NCBI.

Assembly created and it also included to shotgun assembly database. This data can be used to do BLAST analysis to find candidate genes related to abiotic stresses (Zhukov et al. 2015).

3.15.3 Comparative Genome Database

Comparative genome analysis helps improve under researched species using genomic resources of well-studied species. Researchers can generate connection among pea as well as other relevant plants using genetic sequence data. Comparative studies have revealed conservation among pea and many legume crops (Bordat et al. 2011; Leonforte et al. 2013b; Duarte et al. 2014; Sindhu et al. 2014; Tayeh et al. 2015a). Conserved blocks between species helps identify candidate genes for different abiotic stresses like freezing resistance (Tayeh et al. 2013a, b) and various biotic stresses. Basic Local Alignment Search Tool of NCBI database helps to identify gene orthologs in other species (Altschul et al. 1990). Other tools like In-Paranoid (Remm et al. 2001) as well as Ortho-MCL (Li et al. 2003) are also used for comparative genome analysis.

3.15.4 Protein or Metabolomics Database

PlantPReS-Plant stress proteome database was developed by Agricultural institute of Iran (ABRII) which presently contains more than 35,086 entries from 577 articles which are manually curated and more than 10,600 unique proteins related to stress response (as of September 2020) (Mousavi et al. 2016). PSPDB: Plant Stress Protein Database was developed by inserting data which are manually curated proteins from UniProt. It consists of experimentally validated plant proteins related various stresses. It is useful for predicting function of proteins related to stresses (Anil Kumar et al. 2014) (http://www.bioclues.org/pspdb/index.php). Functional analysis of protein can be done using InterPro. It classifies protein into families and predicts domains and other important site by using predictive model which is known as signatures (Mitchell et al. 2019). Pfam is a protein family database used to study protein domains which provides information about protein function. Latest release of Pfam 33.1 contains 18,259 entries as on May 2020 (El-Gebali et al. 2019). Protein Information Resource (PIR) provides resource for protein informatics which supports proteomic research. It maintains three databases i.e., Protein Sequence Database Non-redundant reference database, and integrated database of protein classification database (Wu et al. 2003). The PROSITE database is used to analyze protein domain, families and functional sites. It contains patterns and profiles for protein families and domains which give information like structure and function of proteins (Sigrist et al. 2013). RCSB PDB (Protein Data Bank) is a database which contains structure information for proteins which helps researchers to visualize 3D structures of experimentally determined proteins. Recently, PDB became more user friendly by implementing high-speed NGL Viewer which helps to visualize 3D molecules in any web browser (Rose et al. 2017). Universal Protein Resource (UniProt) is very useful for protein sequence data analysis associate with protein information recourse (Magrane and Consortium 2011). These pathways contain processes like signal transduction, cell cycle, metabolism and membrane transport which is represented in graphical format (Kanehisa and Goto 2000).

3.16 Social, Political and Regulatory Issues

3.16.1 Patent and Intellectual Property Rights

IPR policy based on the concept of intellectual property right should encourage the transformation of scientific research into marketable goods without restricting the exchange of idea between scientist and the public (Lei et al. 2009). Trade related

Dimensions for IPR is an international organization Agreement adopted by the World Trade Organization which comes under General Agreement on Tariffs-Trade (GATT). The US Government developed Plant Patent Act in 1930, mainly to restrict the reproducing, selling, or using the plant by others (Kiran and Pandey 2020). In India, IPR regimes were introduced keeping in mind the interests of farmers and breeders in 2001 through legislation on plant protection verities and farmer's right act, 2001.

A total of 27 pea varieties are registered under PPV&FR. For example, field pea variety Prakash (IPFD 1–10) with its various physiological characters (Varieties and Authority 2001). The IPR regime secures investment returns in the form of royalties or investments for a researcher to pursue research and development in the future. The unethical use of one's research is an crime for which legal action can be taken (Solanki and Chauhan 2020).

3.16.2 Traditional Knowledge

Traditional knowledge is protected globally through various intellectual property right (IPR) laws such as the Copyright Act, the Patent Act, and the Geographical Indication Act. According to the WTO (World Trade Organization), the countries may develop their own *Sui Generis* schemes for the conservation of plant varieties. In India, *sui generis* law such as the Indian Biological Diversity Act, 2002, is a policy with aim to ensure the protection of biological diversity. The overall objectives are to utilizes biological data (Gupta and Prakash 2018).

3.16.3 Participatory Breeding

Participatory plant breeding (PPB) is a framework whereby farmers engage in a plant breeding program with the ability to take decisions and provide inputs at various points during the process. This results in enhanced acceptance of novel cultivars as well as realization of revenue by the farmers. The new efficient PPB approach illustrates high value of information exchange and shared learning between researchers and farmers which create conditions for farmer-researcher cooperation in plant breeding and favors achievement of different agro-ecological and socio-economic goals (Lammerts van Bueren et al. 2018; Annicchiarico et al. 2019).

3.17 Future Perspectives

As the consequence of global warming, various stresses such as elevated heat, water deficiency, extreme cold as well as highly saline soil harm crops and drastically reduce productivity. To overcome this problem, genetic improvement related to production, stress tolerance and quality of seeds is necessary. For this, both traditional and molecular approaches have been used. But these methods haven't solved all the problems, so breeders need to focus on the novel techniques such as genome editing, and precision phenotyping. Recent developments in genome editing technology using programmable nucleases, CRISPR/Cas proteins have numerous benefits for crop improvement.

Pea is generally a spring and winter crop widely grown in many countries like Russia, China, Canada and India. Many abiotic stresses decrease its production. Cold stress is major issue is in Northern-central Europe. Due to this, establishment of routine measurement procedures for efficient integrative analysis for the assortment of cold resistant verities in pea is necessary. New software for automatic analysis of RGB (relative growth rate) image is useful for physiological assortment resembled to shoot development with the efficacy of photosystem II (Humplík et al. 2015). The cultivars grown in colder area revealed higher resistance than the spring grown varieties. For cold tolerant pea breeding, assessment to the increase degree is cold resistance is suggested as significant resources (Zhang et al. 2016). After having the genomic data, the unravelling of functional diversity and establishing a genome enable breeding is a critical step. The recently generated pea genomic data provide tremendous resource for breeders. In two recent impotent projects for pea i.e. GRASP and PeaMUST, extraordinary effort was made to genotype larger pea collections and a large number of traits for the higher yield, symbiosis and resistance to various stresses to various abiotic and biotic stresses were studied using GWAS (Kreplak et al. 2019, 2020). The genetic resources and methods hold promise to enable rapid advances in pea crop improvement.

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Chapter 4
Advanced Breeding Strategies for Abiotic Stress Tolerance in Cowpea

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Abstract Cowpea (Vignaunguiculata L.Walp.) belongs to Papillinoideae tribe of the Fabaceae (Legume) family and is also commonly known as black eyed-pea, crowder pea and southern pea (Singh et al. 1997). Cowpea is an important annual legume crop grown in subtropical and tropical regions (recent reviews in Carvalho et al. 2017). Thus, the crop has worldwide importance. As a legume, cowpea has trifoliate leaves which serve as good fodder but is mostly grown for its edible seeds which are rich in protein, vitamins and minerals. Apart from the seed the green pods can also be used as a vegetable (Hadiet al. 2012). Cowpea seeds have a protein content of up to 25% and are high in micronutrients and essential amino acids like iron and lysine, respectively, which makes them a complementary pulse to the cereal based diets of many consumers in developing countries. Furthermore, cowpea grain is a heart healthyfood with a low fat content of 1.3%, fibre content of 1.8%, and carbohydrate content of 67% made up mostly of complex sugars that are digested slowly by the human gut. In this review we summarize the studies on the abiotic stress tolerances found in this important crop, including those to various environmental or drought limitations such as drought, temperature extremes, and salinity. Germplasm with

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traits for tolerance are described and approaches to classical and molecular breeding of cowpeas given.

Keywords Cowpea · Stress · Tolerance · Genes and diversity

4.1 Introduction

Cowpea (*Vignaunguiculata* L.Walp.) belongs to Papillinoideae tribe of the Fabaceae (Legume) family and is also commonly known as black eyed-pea, crowder pea and southern pea (Singh et al. 1997). Cowpea is an important annual legume cropgrown in subtropical and tropical regions (recent reviews in Carvalho et al. 2017). Thus, the crop has worldwide importance. As a legume, cowpea has trifoliate leaves which serve as good fodderbut is mostly grown forits edible seedswhich arerich in protein, vitamins and minerals. Apart from the seed the green pods can also be used as a vegetable (Hadi et al. 2012). Cowpea seeds have a protein content of up to 25% and are high in micronutrients and essential amino acids like iron and lysine, respectively, which makes them a complementary pulse to the cereal based diets of many consumers in developing countries. Furthermore, cowpea grain is a heart healthyfood with a low fat content of 1.3%, fibre content of 1.8%, and carbohydrate content of 67% made up mostly of complex sugars that are digested slowly by the human gut.

Plant parts of cowpea after grain harvest including vines and old leaves are used as fodder while tender leaves and some snap pods types are used as vegetables in certain countries (Abbas et al. 2013). The leaves of cowpea make an excellent feed stock and can be mowed into a nutrient rich hay or kept as a valuable silage, that is equivalent to alfalfa but adapted to hotter climates. The growth of plant differs in their varieties, it may be short, bushy type and others are vinyand tall or rambling across the ground (Singh et al. 1997). Cowpea plants reach aheight of 25–30 cm tall when bush types are grown in normal conditions, but vines can be 3 m tall. Growth of root generally occurs within the topsoil layer, but in condition of drought it can grow taproots that areat least 2.5 m long to reach for moisture deeper in the soil. Roots of cowpeas usually have more nodules compared to soybean roots, with *Bradyrhizobiuim* spp. As the nodule bacterial symbiont.

Flowers of cowpea have extra floral nectaries which attract pollinators. Pores on leaves and stems of leaves, both release nectar and attract the beneficial insects. Cowpea flowers are mostly self-pollinating but can vary in color from white to pink, pale blue or purple. The flowers are butterfly-shaped with lobes of the standard fused and lateral wing petals shorter compared to the upper fused petal. A keel is arranged in the middle of the flower and contains the staminal column and pistil that when pollinated develops into an elongated fruit, called a pod. Each ovary contains multiple ovules and as a result the pods contain up to a dozen or more seed. The flowers and pods are arranged in intermediate inflorescences in alternate pairs. Flowers open in the early time of day and close at midday. Pollinating insect activities are beneficial for increasing the pod set and seed (McGregor 1976). The pods of cowpea vary in

size, shape, texture and color. They may be erect, coiled, elongated and straight or crescent shaped. Seeds in dry grain types are developed in 8–10 cm long, round, slender, two-valved pods growing from the leaf axils. Pods are usually greenish yellow when ripe but also brown or purple. Pods usually have 8–20 seeds per pod, pods vary in size, shape and color and they are kidney shaped. Covering of seed may be smooth or wrinkled with white, green, brown, red, black eyed or mottled (Aveling 1999).

Around the world, cowpeas are grown on more than 12.5 million hectares with an annual production of more than 3 million tons in Sub-Saharan Africa alone (Singh and Matsui 2002). Cowpea is a warm season legume, adapted to high temperatures (20–35 ℃). It is fast growing crop that produce 2500–4500 lb/acre/yr dry matter (Kunert 2017). It can grow well in varieties of soil textures, like heavy clays and sandy soil. In terms, of abiotic stresses, cowpea does not like extremely acid soils, performing better in slightly acid to alkaline soil (pH 5.5–8.3). The cowpea plant is well known for growing under extreme drought condition. It has little tolerance towards salinity but is tolerant for low phosphorus and other nutrients especially on sandy well drained soils where it searches for minerals deep in the soil (Valenzuela and Smith 2002). Despite its sensitivity to acid soils, cowpea tolerates high content of aluminium perhaps due to organic acid exudation by the roots. As a symbiotic nitrogen-fixing legume, cowpea is adapted to low nitrogen soils. Poor soil tolerance is mostly mediated by root characteristics while drought tolerance is mostly mediated by the whole plant and this along with the impact of the rhizosphere and weather conditions are discussed in the sections below.

4.2 Available Germplasm

Total number of genetic resources available in a species can be termed as germplasm. Germplasm resources are the most important component of crop improvement programmes. Breeding strategies adopted in different crop plants is greatly influenced by the availability of germplasm resources. The conservation of variability present in the germplasm is of vital importance for present as well as our future generations. Various research organizations across the globe are involved in germplasm collection, conservation, characterization, documentation, and distribution. Cowpea being one of the most important arid legumes possesses large amount of variations for morpho-phenological traits. In India ICAR-NBPGR is the nodal agency for introducing trait specific novel genetic resources, through which various Indian institutes have been able to import a total of 4922 accessions for various research activities in the past 30 years (Tripathi et al. 2019). Organizations such as IITA, Nigeria; Institute of Plant Breeding, University of Philippines; Department of Plant Industries, Australia; Plant Germplasm Quarantine Centre, Beltsville, Maryland, USA; Regional Plant Introduction Station, Georgia, USA; University of California, Riverside, California, USA and USDA-ARS, US have contributed cowpea germplasm accessions. Details of Cowpea accessions maintained in various institutions across globe has been given in

Table 4.1 Details of Cowpea accessions maintained in various institutions across globe

Sl No	Country/Institute	No. of cowpea accessions
1	Angola (SADC)	172
2	AVRDC-Taiwan	322
3	Belgium	331
4	Benin	155
5	Cote d'Ivoire	126
6	Germany	291
7	IITA, Nigeria	15004[a]
8	India (NBPGR)	3648[b]
9	Kenya	875
10	Namibia (SADC)	57
11	Nigeria	384
12	Russia	1945
13	South Africa	886
14	South Korea	910
15	Spain	466
16	Swaziland (SADC)	45
17	Tanzania	386
18	Zambia (SADC)	305
19	United States–California (UC Riverside)	5600
20	United States–National (USDA)	8255[a]

Source Global Crop Diversity Trust (2017), Tripathi et al. (2019)

(Table 4.1) and List of trait specific accessions identified in cowpea is presented in (Table 4.2).

4.3 Abiotic Stress Tolerance Related Traits

4.3.1 Root Characters

Many types of soil have different hurdles to overcome when trying to grow crops in the most efficient and productive manner. Therefore, research needs to target specific soil types and/or specific aspects of soil composition to find specific management practices and plants that tolerate such deficiencies. For this an understanding of the rhizosphere and root system of a crop is key. Cowpea root system are dense and well-developed and has a beneficial effect on the structure and tilth of the topsoil

Table 4.2 List of trait specific accessions identified in cowpea

Trait	Accession
Early flowering (<50 Days)	EC723666, EC723684, EC723690
Extra early (50% of the plants produce their first NBPGR, New Delhi flowering 37 days after sowing)	EC528457
Early maturity (<87 Days)	EC724381, EC723746, EC723797
Erect habit (<66 cm)	EC723894, EC723684, EC724381
More number of pods per plant	EC724307, EC724366, EC725116, EC724547, EC724770
Higher pod length	EC723681, EC725162, EC724327, EC724045, EC724536
More number of seeds per pod	EC725164, EC723741, EC723971, EC725180
Higher protein content (>25%)	IC536626, IC536637, IC397807
Fodder type	EC723771, EC725106, EC723662, EC723995, EC723908
Large seeded and stay green	IC202793

Source (ICAR-NBPGR 2016; ICAR-NBPGR 2018; Rana et al. 2016; Tripathi et al. 2019)

layer. Most root growth occurs within the topsoil layer but, in drought conditions, a long taproot can grow for reaching the deeper moisture in the soil profile.

These characteristics furnish cowpea plants with a high resistance to drought in comparison with other legumes (Hall 2012). Scanning for root length, root width or diameter and branching of main or lateral root scan be used to identify differences in growth of abiotic stress treatments whether in soil or hydroponically (Cancado et. al. 1999; Lopez-Marin et al. 2009).

4.3.2 Heat and Cold Tolerance

High temperature damage to reproductive processes of cowpea occurs when minimum night air temperatures are greater than 20 °C. The extent of damage is strongly influenced by photoperiod. Genes conferring heat tolerance under hot long-day environments have been identified in cowpea, and breeding lines with this trait have been developed. Genetic variability within cowpea germplasm for heat tolerance has not been assessed in short days as these are typical of cooler seasons. Development of heat-tolerant cultivars is the major practical approach to reducing losses in grain yield due to heat (Hall 1992).

For most cowpea production regions in the world, sowing date cannot be varied to escape heat at flowering. Sowing must be done either at the onset of the warm season (in the subtropics), or at the onset of the rainy season (in the tropics) and can result in high temperatures during the flowering period. Cowpea lines differ in their response

to high temperatures during reproductive development under long days (Patel and Hall 1988).

Strong tolerance to heat-induced floral bud suppression and low pod set was discovered in cowpea accessions Prima and TVu4552 (Warrag and Hall 1983). These breeding lines have exhibited no suppression of floral bud development and possess higher pod set (pods per peduncle) and total number of pods per plant under extremely hot, long-day, field conditions (41–50 °C/22–27 °C daily maximum/minimum air temperatures, respectively, and 14–15 h day length during floral bud development and pod set), than commercial California cowpea cultivars and other accessions (Ehlers and Hall 1996). The heat-tolerant breeding lines have out yielded a commercial cultivar by two- to five-fold in two years of tests in a scorching hot field environment in the California low-elevation desert (Ismail and Hall 1998).

Day length can influence the responses of cowpea to heat (Ehlers and Hall 1996). The effects of high temperatures on pod set under short days (day length of 12.5 h or less) have been described only for two lines grown in growth chambers and in a preliminary greenhouse study (Hall 1993). Little is known about the performance of the heat-tolerant breeding lines under hot short-day conditions typical of the major tropical cowpea production regions (Nielsen and Hall 1985).

4.3.3 Drought Tolerance

Drought tolerance is defined as the ability of plants to live, grow and yield satisfactorily with limited soil water supply or under periodic water deficiencies (Ashley 1993). According to Mitra (2001), the mechanisms that plants use to cope with drought stress can be grouped into three categories, namely: drought escape, drought avoidance and drought tolerance. However, crop plants, especially legumes like cowpeas use more than one mechanism at a time to cope with drought (Hall 2012).

Drought escape is defined as the ability of a plant to complete its life cycle before serious soil and plant water deficits occur. This mechanism involves rapid phenological development (fast seedling growth, early flowering and early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water deficit) and remobilization of pre-anthesis assimilates to developing pods. Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil-moisture. Plants develop strategies for maintaining turgor by increasing root depth or developing an efficient root system to maximize water uptake, and by reducing water loss through reduced epidermal (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or a smaller leaf area that lowers evapo-transpiration (Mitra 2001). Drought tolerance is the ability of plants to withstand water-deficit with low tissue water potential. The mechanisms of drought tolerance are maintenance of turgor through osmotic adjustment (accumulation of solutes in the cell), increased cell elasticity, decreased cell size and desiccation tolerance by protoplasmic resistance.

Selection for early flowering and maturity and yield testing of breeding lines under drought conditions has been successful in developing cowpea cultivars adapted to low rainfall areas (Cisse et al. 1997; Hall 2012). The different screening techniques that were tested included: the antioxidative response in the form of superoxide reductase (SOD), glutathione reductase (GR), ascorbate peroxidase (AP), proline accumulation, 2,3,5-triphenyltetrazolium chloride (TTC) assays, early drought screening at the seedling stage (wooden box technique), cell membrane stability (CMS), relative water content (RWC), leaf water potential (LWP), leaf area, chlorophyll a and b and carotenoid content and chlorophyll fluorescence (JIP test). Contrary to the results of Carvalho et al. (1998), RWC was an excellent parameter to discriminate tolerant genotypes under water stress in cowpea (Slabbert et al. 2004).

4.3.4 Salinity Tolerance

Salinity is a major limiting factor for crops productivity on more than 20% of cultivated land worldwide. Adverse effects of salt stress are ascribed to osmotic stress, ion toxicity and production of reactive oxygen species. To cope with salt stress, plants undergo various biochemical and physiological changes, including: biosynthesis of compatible solutes and osmoprotectants, ion homeostasis and compartmentalization, control of mineral uptake and transport, selective accumulation or exclusion of ions, generation of nitric oxides, changing hormone levels and enhanced antioxidative systems. Salt stress has these same harmful effects on the biochemical, physiological and growth attributes of cowpea plants.

To mitigate deleterious impacts of salinity on crop plants, researchers have attempted hormone and genetic approaches. In the first case, seeds soaked in 50 μM MeJA had increased salinity tolerance through accumulation of proline, soluble sugars and proteins while improving chlorophyll value and net photosynthesis (Sadeghipour 2017). Plant breeding has been used for, exogenous application of some organic and inorganic substances to seeds/seedlings which might be an important strategy for improvement of salt tolerance.

Cowpea has a moderate resilience to high salt soils, with a more noteworthy resistance than corn yet not as much tolerance as wheat, grain, sugar beet, or cotton (Hall and Frate 1996). Fortunately, a few accessions of cowpea are adjusted to adapt to this abiotic stress, as well as drought and elevated temperature and solar radiation (Silveira et al. 2003) which alone or together, can impel oxidative harm to the plant (Foyer and Noctor 2000). Some plant species developed in selenium-enriched media have demonstrated upgraded tolerance to salinity and other abiotic stresses (Djanaguiraman et al. 2005; Kong et al. 2005; Hawrylak-Nowak 2009).

4.3.5 Herbicide Tolerance

Herbicide resistance traits can be efficiently introduced to the cowpea gene pool, enhancing its germplasm and other agronomically important traits for the improvement of cowpea cultivar using immature cotyledon explants. The use of the bar gene encoding herbicide resistance provides an efficient screening of transgenic cowpea plants as a selection marker as well as an efficient means of weed control (Aasim et al. 2013).

4.3.6 Nutrient Use Efficiency

Nutrient use efficiency is typically divided into two interactive components: the efficiency of nutrient acquisition (i.e., the amount of nutrient taken up by plants in relation to nutrient supply) and the efficiency of nutrient utilization, which informs the biomass produced by the unit of nutrient incorporated by plants. Nutrient use efficiency (NUE) is a measure of how well plants use the available mineral nutrients. It can be defined as yield (biomass) per unit input (fertilizer, nutrient content). NUE is a complex trait: it depends on the ability to take up the nutrients from the soil, but also on transport, storage, mobilization, usage within the plant, and even on the environment.

4.3.7 Aluminium Toxicity

Aluminium stress is important for cowpea production in many parts of the world where topsoil or sub-soils is acidic including much of Sub-Saharan Africa, certain parts of Asia including southern China and on most cowpea production sites in North and South America or the Caribbean (Kochian 1995). Aluminium toxicity is a limitation in the South-eastern states of the USA, on the islands of Cuba and Hispaniola, in certain highland areas of Central America and in the Andean region of South America as well as in central and southern Brazil (Rao et al. 2008). Root growth is inhibited by phytotoxic Al^{3+} ions. This limitation due to aluminium toxicity can be resolved by increasing pH through liming but most subsoils in these areas remain with lower pH that still prevent subsoil penetration by cowpea roots to obtain nutrients or water (Ryan et al. 1993). Addressing aluminium stress is important for growing cowpeas as it is a low input crop in these regions. Currently the supply of aluminium tolerance varieties with companies or academic institutions is low. Breeding programs should aim to develop and release cultivars having the ability to adapt to low pH soils with high Al saturation, which unfavourably affects crop production.

4.4 Sources of Abiotic Stress Tolerance Genes

Wild species consists reservoir of tolerant genes for different biotic and abiotic stresses. Bringing resistant genes into the cultivated background is limited by crossing barriers. Initiation of pre-breeding work is essential for bringing tolerant/resistant genes from wild to cultivated species, resulting in directed advancement in the crop improvement program. Cowpea belongs to the genus *Vigna,* the genus *Vigna* consists of several important species which can be a potential source of resistance to different abiotic stresses which can help in breeding climate-smart cowpea cultivars for sustainable agriculture.

Genus *Vigna* is divided into five subgenera: *Ceratotropis, Haydonia, Lasiospron, Plectrotropis,* and *Vigna.* Out of which 3 of the subgenera include crop species: Plectrotropis and *Vigna*, which originated in Africa, and Ceratotropis, which originated in Asia. Subgenus *Vigna* comprises of 3 sections namely *Catiang, Macrodontae,* and*Reticulatae.* Cowpea belongs to section *Catiang,* species *unguiculata*, sub-species *unguiculata. V. unguiculata var. spontanea* (formerly var. *dekindtiana*) is believed to be the progenitor of cowpea (Boukar, 2013).

Ng and Marechal (1985) categorized cultivated cowpea into four groups, namely *Biflora, Sesquipedalis, Textilis*and*Unguiculata.* Group *Sesquipedalis*, consists of yard-long bean (*V. unguiculata* group *sesquipedalis*), which is mostly cultivated in Asia, and grain type cowpea falls in (*V unguiculata* group *unguiculata*), which was domesticated in West and Central Africa. Southern Africa is centre of origin for wild cowpea as proposed by Padulosi and Ng (1997). Wild accessions are found for *V. unguiculata*, and for four related species (*V. dekindtiana, V. stenophylla, V. tenuis* and *V. vexillata*). Crossing between most of them does not result in production of fertile and viable progeny although *V. vexillata*is considered to beamong the most closely related to cultivated cowpea (Garba and Pasquet 1998) and a source of high resistance to various abiotic stresses, such as waterlogging and alkalinity (Marubodee et al. 2015) (Table 4.3).

4.5 Genetic Diversity Analysis

Genetic diversity refers to magnitude by which individuals in a group differ among themselves (Hintum 1995). Genetic diversity is considered pre-requisite for crop improvement programs which either exploit the prevailing variation or create them. The diversity in the cultivated crops indicate richness of the gene pool and eases breeder's search and in turn its usability. It is a valuable resource for plant breeders in crop productivity improvement (Wamalwa et al. 2016) and other related economic traits. Cowpea accessions have been reported to exhibit lower genetic diversity (Karuma et al. 2008; Asare et al. 2010; Wamalwa et al. 2016; Sarr et al. 2021) due to several reasons discussed below. In addition, lack of population structure or correlation between accessions and their geographical region of collection was also

Table 4.3 Sources of resistance for various abiotic stresses in cowpea

Abiotic stress	Source	Reference
Drought tolerance	TVu 11,979, TVu11986, TVu 12,349, Dan' Ila, and IT90k-59–2	Watanabe et al. (1997)
	VCP 16 and CO 6	Anantharaju et al. (2008)
	IT93K-503–1	Muchero et al. (2008)
	Kanannado, Danila, IT07K-297–13, IT03K-378–4, and Aloka local	Ismaila et al. (2015)
	Gorom local (Go), Mouride (Mo), TN88-63(TN),	Hamidou et al. (2007)
	Apagbaala, IT 87D-885	Alidu et al. (2013)
	IT96D-604	Matui and Singh (2003)
	IT89KD-374–57, IT88DM-867–11, IT98D-1399, IT98K-131–1, IT97K-568–19, IT98K-452–1, and IT98K-241–2,	Timko and Singh (2018)
	Acc1257, Acc1168, Acc2355, IT96D-602and Acc5352	Nkoana et al. (2019)
	C11, C18, C44, C46, C47, C50 and C54 (tolerance at germination)	Carvalho et al. (2019a, b)
	PI339600, PI527263, PI527302, PI582793, PI582867, SARI-6–2-6	Yahaya et al. (2019)
	C47 (Iran), C56 and C11(Portugal)	Santos et al. (2020)
Heat tolerance	IT 07–274-2–9, IT 90 K-59, RV 344 and 835–911	Nkomo et al. (2020)
	IT93K-452–1, IT98K-1111–1, IT93K-693–2, IT97K-472–12, IT97K-472–25, IT97K-819–43 and IT97K-499–38	Timko and Singh (2018)
	TVu 4552	Patel and Hall (1988)

(continued)

Table 4.3 (continued)

Abiotic stress	Source	Reference
	EC496737 California black eye reg. No. (CV-167)	Tripathi et al. (2019)
Salt tolerance	Hai Jiang San Hao and Green Super	Islam et al. (2019)
	PI582422, 09–529, PI293584, and PI582570	Ravelombola et al. (2017)
Multiple abiotic stresses	UCR-193, MPE and CB-5	Singh et al. (2010)
P use efficiency	IT89KD-288	Boukar et al. (2018)
Low P tolerance with enhanced N fixation	IT89KD-374–57, IT90K-372–1-2, IT98D-1399, IT99K-1060, IT97K-568–19, IT97K-568–11, IT00K-1148, IT97K-1069–6, IT03K-314–1 and IT03K-351–2	Timko and Singh (2018)
Al toxicity tolerance	Dixie lee, queen anne	Hickok and Blair (in prep)

reported (Mafakheri et al. 2017; Gomes et al. 2020; Sarr et al. 2021). In contrast, accessions varied in accordance with the geographical regions in collections derived from China (Chen et al. 2017). That study consisted of wild and weedy relatives of cowpea, which were distinctly grouped with few intermediate cultivated African cowpea germplasm from Senegal showing admixture.

Analysis of marker variance (AMOVA) indicated prevalence of higher variation among individuals within regions (Chen et al. 2017; Gomes et al. 2020; Sarr et al. 2021) rather than inter-population variations. Probable reasons for this may be local exchange of the germplasm among the farmers, seed trades (Baudoin and Maréchal 1985), interventions of seed companies and agricultural extension services (Sarr et al. 2021). Evolutionary constraints might also have contributed in lowering diversity leading to genetic homogeneity. Prevalence of genetic bottlenecks in artificial or natural selection and favoring few varieties over remaining heterogeneous individuals can best explain absence of variation.

Further, domesticated variation might have been well preserved without much addition due to cultivated Cowpea being predominantly self-pollinated (Padulosi and Ng 1993). The region-specific domesticated traits have spread across germplasm, which has manifested as absence of region wise population structure, due to considerable allelic migrations between the cultivated forms (Sarr et al. 2021). Alternatively, single domestication event involved in evolution of this crop might have added to lower prevalence of genetic diversity as well (Asare et al. 2010; Wamalwa et al. 2016).

4.5.1 Phenotype-Based Diversity Analysis

To conserve and utilize germplasm collections efficiently, quantification of diversity prevalent is as important as characterization of the accessions (Terzić et al. 2020). Several attempts have been made in clustering the genotypes based on phenotypic data using various statistical tools like distance matrices, principle component analysis, principle coordinate analysis etc. Grain legumes being important protein supplements in African countries, most of the studies related to cowpea germplasm accessions are from African or sub-African countries. Yahaya et al. (2019) used primary yield variables and secondary variables such as drought intensity index (DII), drought susceptibility index (DSI), drought tolerance index (DTI), geometric and mean productivity as well as yield reduction rate (YRR) to evaluate cowpea varieties in Ghana.

Similarly, germplasm of Indian origin has also been subjected to diversity studies. Seed yield as major contributing trait towards diversity among cowpea from Indian and Nigerian regions was reported by Vishwanatha and Yogeesh (2017) in these lines. Other South and West Asian genotypes have been analysed. For example, cowpea germplasm lines from Iran were clustered with 17 quantitative morphological traits recorded under water stress and irrigated conditions fell into four clusters. However,

Table 4.4 List of traits and specific cowpea accessions with advantages for drought breeding

Trait	Accession
Early flowering (<50 Days)	EC723666, EC723684, EC723690
Extra early (50% of the plants produce their first flowering 37 days after sowing)	EC528457
Early maturity (<87 Days)	EC724381, EC723746, EC723797
Erect habit (<66 cm)	EC723894, EC723684, EC724381
High number of pods per plant	EC724307, EC724366, EC725116, EC724547, EC724770
High pod length	EC723681, EC725162, EC724327, EC724045, EC724536
High number of seeds per pod	EC725164, EC723741, EC723971, EC725180
Higher protein content (>25%)	IC536626, IC536637, IC397807
Fodder type	EC723771, EC725106, EC723662, EC723995, EC723908
Large seeded and stay green	IC202793

the grouping of the genotypes was not similar in water deficit and well-watered conditions genotypes with higher seeds per plant and test weight were considered superior under drought conditions (Mafakheri et al. 2017). Meanwhile, other researchers considered traits related to seedling establishment as important in grouping cowpea accessions to drought tolerance (Hall 2012; Emanoela et al. 2018). Based on germination, growth and phytomass accumulation, genotypes under stress and non-stress were grouped separately indicating substantial difference between the genotypes for response to water deficit. Four genotypes among nine tested were found to be most tolerant to water stress. Tripathi et al. (2019) identified multiple sources of traits that could be useful for drought tolerance breeding (Table 4.4).

For African growing conditions, a collection of cowpeas from Ghana, Benin and Nigeria was used for morphological diversity study considering 38 qualitative and quantitative traits to categorize 47 cowpea accessions. Bootstrapping of the nodes indicated prevalence of two main clusters (Kwadwo et al. 2020). However, phenotypic expression recorded in single season are less reliable due to complex genotype-by-environmental interactions (GEI). Multi-season/location-based diversity analysis of morphological traits have been reported in other parts of Sub-Saharan Africa (Bozokalfa et al. 2017; Nkhoma et al. 2020). On-farm trials for drought tolerance were conducted at three locations in northern Ghana in the Savannah ecological zone (Yahaya et al. 2019) with average drought intensity index of 0.61 and significant differences in days to flowering, days to maturity, yield per plant and yield per hectare. Seven genotypes out of 240 in an augmented design and six out of 50 in a randomized complete block were the most drought tolerant.

4.5.2 Genotype-Based Diversity Analysis Based on Molecular Marker Studies

Molecular marker based genetic diversity is being studied since discovery of markers. Isozyme based diversity was attempted in cultivated species of cowpea by Reis and Frederico (2001), where most of the markers except for esterase enzyme system were monomorphic. Further, DNA marker-based diversity analysis was studied by Sharma et al. (2018); Ba et al. (2004) where Random primers were used for PCR amplification of Cowpea genome. Other popular DNA marker systems like AFLP or Amplified Fragment Length Polymorphism (Kwadwo et al. 2020), DNA Amplification fingerprinting (Spencer et al. 2000) and combination of these have also been used. By the turn of the century, SSRs or simple repeat markers were used widely in analyzing genetic diversity of cowpea (Gomes et al. 2020; Chen et al. 2017; Wamalwa et al. 2016) sometimes in combination with other marker systems (Gillaspie et al. 2005) or morphological characteristics (Mafakheri et al. 2017).

Single nucleotide polymorphism (SNP) markers are the newest type of marker system developed and they are believed to be ideal for genetic diversity studies due to their wide genome coverage and amenability for automation. Nkhoma et al. (2020) used around 14 K SNP in studying diversity in germplasm collections of cowpeas while around 6 K SNPs were used by Ketema et al. (2020) for classification of 357 accessions. However, there are other instances where SNPs are used in gene pool analysis of cowpea by Huynh et al. (2013).

Genetic diversity prevailing in the germplasm manifests as a phenotypic variation or allelic variations. Often, there exists discordance between the diversity analysis considering phenotypic and molecular markers. These inconsistencies may be due to molecular markers' ability to capture synonymous and other subtler genomic variations that may not express phenotypically (Nkhoma et al. 2020). Due to this discord, combined consideration of genotypic and phenotypic matrices, forming joint matrix, has been recommended to precisely capture genetic diversity (Singh et al. 2013; Sartie et al. 2012). The joint matrix is reported to increase the precision of dissimilarity estimates by 1.5 times in comparison to individual matrices (Alves et al. 2013).

4.5.3 Molecular Mapping in Cowpea for Abiotic Stress Resistance

Molecular mapping eases crop improvement of the traits by providing markers associated with the trait of interest. Linkage mapping and association mapping are the available tools for mapping the genomic regions of economic importance onto the established linkage map. Cowpea, a diploid with $2n = 22$, has 11 linkage groups. These linkage groups receive markers and facilitate trait associations, as researchers attempt to map traits. As an initial procedure for mapping, selection of donors or contrasts for

linkage map construction is crucial. The germplasm resources conserved in national and international repositories serve as basis of this selection. In contrast, association mapping uses historical linkage disequilibrium prevailing in the germplasm so conserved, for establishing marker trait associations. Several markers are used for mapping traits of interest in cowpea and are reviewed by Boukar et al. (2016). In Cowpea, first linkage map was developed by Fatokun et al. (1993) consisting of 89 marker loci bracketing RFLP, RAPD, cDNA based markers and a morphological marker. However, attempts to map QTLs governing resistance to abiotic stresses are relatively recent. Genomic regions governing tolerance to seedling drought stress was mapped to 10 QTLs by Muchero et al. (2009), of which some of the QTLs coincided with that of stay green trait. Further, RFLP markers alone were used to map QTLs governing seedling drought stress by the same group, using RILs derived from same F_2 population. This attempt identified seven QTLs responsible for drought stress (Muchero et al. 2010).

Heat induced browning of seed coat is an alternative measure of response to heat stress. In this context, three QTLs were identified in RIL population using SNP markers for response to heat stress (Pottorf et al. 2014). In addition, plants manifest significant decrease in pollen fertility and there by seed set on exposure to heat stress. This phenomenon as a trait was mapped on to five regions on the cowpea genome by Lucas et al. (2013) employing SNP markers. All the QTLs reported thereof were major, governing significant phenotypic variance (11.5–18.1 per cent). Syntenic studies of the mapped regions with soyabean indicated prevalence of genes governing heat shock proteins, heat shock transcription factors, and proline transporters (Lucas et al. 2013). Mapping attempts to other abiotic stresses, however, are less, limiting availability of markers in improvement of the crop.

4.5.4 Molecular Breeding

Molecular mapping of crops opens of arenas for quick improvement of target traits through assistance of molecular resources. Conventionally, transfer of traits from a donor to agronomically superior lines, with minor defects, to derive isogenic lines requires considerable resources and time. In contrast, marker aided crop improvement, manifesting as MABC, MARS, F_2 enrichment can ease the selection procedures as well as considerably decrease time and resource requirements. Prime requirement of such experiments is marker(s) preferably genic, if not flanking the target trait/QTLs close enough to avoid false selections. However, of-late genomic selections are employed to bypasses the cumbersome process of QTL mapping and validation. It considers major and minor QTLs together in efficient selection and imparting significant genetic gains.

In cowpea, most of the marker aided back crosses are attempted and implemented through CGIAR-GCP-TLI (Consultative Group of International Agricultural Research-Generation ChallengeProgramme-Tropical Legumes I) project at IITA and NARS centers in collaboration with the University of California, Riverside (UCR).

However, there are less reports on improvement of cowpea for abiotic stress tolerance, while improvement for tolerance to striga, increase in seed size and grain quality are attempted. In contrast to MABC, marker assisted recurrent selection is used for improvement of drought tolerance. In this attempt, 177 lines were fixed for favorable alleles at seven QTLs using SNP markers. Further, these lines were used for deriving advanced breeding lines. Similarly, MARS has been employed to improve lines for heat tolerance and other agronomic traits at Eduardo Mondlane University. At ISRA, Senegal, there are attempts to improve populations for drought tolerance and other biotic stresses (Chamarthi et al. 2019). However, genomic prediction and selection studies for abiotic stress in cowpea are limited though they are considered to have edge over marker assisted breeding in realizing superior genetic gains.

4.5.5 Genomics Assisted Breeding

On considerable research in Cowpea, there are sufficient genomic resources available for crop improvement. Advances in genomic tools have opened up areas aiding precise pointing at genomic regions responsible for various economic traits of interest. Genomic approaches have been widely used in cowpea for knowing the functions of mapped regions, assigning them onto chromosomes, identifying expressed sequences through transcriptome, proteome and metabolome analysis. Of-late, phenomics has been extensively used for phenotyping traits of economic importance for mapping or screening studies. These tools can very well boost the breeding progress in any crop with no exception to Cowpea.

Comparative genomics approaches have enabled precise identification of origin of Cowpea and unambiguous identification of progenitor as *V. unguiculata* ssp *dekindtiana* (Coulibaly et al. 2002). Further, several attempts to map QTLs and oligo genes in Cowpea have used both random and sequence based molecular markers. As a result, drought tolerance QTLs were placed onto linkage group 10, similar to other genomic regions governing several biotic stress tolerance (Diouf 2011). Attempts to deduce functional meaning of genomic sequences in Cowpea have yielded several successful results. Transcriptomic approaches, as a branch of functional genomics, have unravelled mechanisms underlying tolerance to drought stress in Cowpea. The mechanisms that the crop has evolved to prevent lipids and proteins degradation, generation of reaction oxygen species (ROS) like superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) are well understood through transcriptomics. It has even helped identification and characterization of transcripts for drought, salinity and heat stress (Iuchi et al. 2000). Further, differential expression of transcripts for heat stress and their putative roles in heat tolerance are also reported by Carvalho et al. (2006). Similarly, proteomics and metabolomics can further aid in arriving at candidate genes responsible for abiotic stress tolerance, thereby opening up scope for vector based direct transfer of traits. However, phenomics can only aid in precision of phenotyping traits either for mapping or germplasm screening studies, which is the basis for most of the plant breeding works. In Cowpea, there is priming of use of

phenomics for understanding root characters (Burridge et al. 2020; Das et al. 2015), though not for abiotic stresses as such, which can be related to drought and related mechanisms.

4.6 Classical Genetics and Traditional Breeding for Abiotic Stress Tolerance

Food security is of concern in this era of technological and industrial revolution. In the days to come, challenge would be to increase the food production in a given or still decreasing cultivable holdings, threatened by developmental activities, desertification, salinization and emerging diseases. Apart from biotic agents, abiotic factors are also expected to impede realization of potential crop yields.

Plant breeders need affordable and scalable solutions to achieve this sustainability (Janni et al. 2020). In these lines, sources of tolerance to abiotic stress can be harnessed from natural and artificial mutations, search in the germplasm collections through improved phenotyping facilities and recombining the existing variability to arrive at transgression lines to combat the changed agricultural growth conditions. In the following sections, abiotic stress and impacts thereof are discussed along with traditional and marker assisted breeding perspectives in combating them.

4.6.1 Heat and Cold Tolerance

Heat and cold stresses in plants impair their physiological, biochemical and other processes which involve heat sensitive compounds involved in normal functioning of the cell including protein metabolism and enzyme activity. At a macro level, this manifests as yield and grain or fodder quality losses (Jha et al. 2017).In extreme cases, heat stress intervenes with photosynthetic machinery, respiration process, reduces stomatal conductance, inhibits TCA cycle activity, and increases reactive oxygen species (Way and Yamori 2014).

Reproductive phase, being most economically viable for food production, is most vulnerable to heat stress. Though apparently flower abscission, anthesis impairment, low pod set and grain filling are observed, flower bud formation and opening, pollen viability and germination, pistil viability, and embryo development are also affected.

In addition, scorching of plant parts render the source to be incapable of photosynthate supply and stunts the overall plant development. It is reported that high night temperature lowers sugars in peduncles leading to poor pod set and causes substantial damage to reproductive parts in heat sensitive cowpea genotypes (Ahmed et al. 1993; Mutters et al. 1989). Molecular markers have been found for preventing heat-induced, seed coat discoloration (Lucas et al. 2013) an important trait for light colored cowpeas.

Understanding the genetics of the trait under interest is of prime importance in designing strategies in improving the trait. Heat tolerance in Cowpea is reported to be governed by single dominant gene (Marfo and Hall 1992) based on heat tolerant (Prima and TVu 4552) and heat-sensitive (Bambey 23 and Magnolia 7964) cowpea genotypes. However, during flower bud development stage, it is reported by same authors to be governed by single recessive gene. However, genetic control might be of complex governance involving GEI with major and minor genomic regions (Jha et al. 2017).

Cowpea genotypes tolerant to heat stress, traits involved in conferring heat stress and their origin are provided in Jha et al. (2017). In breeding for heat stress, several segregating populations has been developed using tolerant and susceptible genotypes. There is report of development of multi parent derived populations in simultaneously expanding genetic base of Cowpea germplasm and imparting resistance to heat stress (Huynh et al. 2019; Olatoye et al. 2019).

Similarly, cold stress has negative impact on germination and establishment of Cowpea (Islam et al. 2006). Screening of germplasm indicated prevalence of variability and tolerant genotypes in cowpea for low temperature stress. During characterization of entire cowpea germplasm (3,720 accessions) at ICAR-NBPGR in India, a unique accession in cowpea with dense pubescence was identified (Tripathi et al. 2020). The presence of trichomes are reported to increase tolerance towards freezing in plants. They can help reduce evaporation by protecting the plant from wind and heat (Serna and Martin, 2006).

QTLs have been identified for heat tolerance in cowpea suggesting the inheritance is polygenics (Lucas et al. 2013). However, the combination of heat and drought has mostly been analysed in the dry Western growing environment of Imperial Valley of California in the United States (Fery 1990; Hall, 2012). Therefore, these QTL should be confirmed in areas outside of this environment, such as in South Asia or West Africa. No QTL for cold tolerance have been identified as this trait is not important in most cowpea growing regions. Both higher and lower temperatures affect the genetic control of flowering time (Andargie et al. 2013).

4.6.2 Root Characters and Drought Tolerance

Drought in crop plants is a common phenomenon in tropical countries (Hall 2012; Carvalho et al. 2017, 2019a, b). It is characterized by water stress either due to lack of rainfall or improper irrigation practices. In crop plants, water deficit causes flower and fruit drop with no exception to Cowpea. Crops, when encounter water stress at critical stages, exhibit impaired yield (Omae et al. 2007).

Cowpea has flowering and pod formation as critical periods which when coincides with water deficit, leads to severe yield and quality losses. However, Ndiso et al. (2016) commented that mild water stress at vegetative and flowering stages increases grain yield grain cowpea while severe stress impairs growth parameters and chlorophyll content. Yet another consequence of drought in cowpea plants is

reduced transpiration in leaves and thereby increasing the leaf temperature (Miranda et al. 2012). As a strategy, plants ought to put on deep roots to wetter regions of soil, rather than at surface, to absorb moisture. Drought tolerant genotypes usually have deeper root systems compared to susceptible varieties (Guimarães et al. 1996; Hall 2012). Other root traits like root length, root density, root volume and root biomass are also considered in selecting drought tolerant varieties (Asfaw et al. 2012).

Attempt to dissect the genetics of drought tolerance in cowpea revealed predominance of quantitative governance with major and minor contributing genomic regions (Hall 2012). Combining ability analysis for pod yield under drought stress indicated prevalence of additive gene action (Alidu et al. 2013). In contrast, Mwale et al. (2017) opined prevalence of both additive and non-additive gene actions yet non-additive governance being predominant in seed yield under drought stress indicating dependencies of gene actions on genetic background of the varieties. Thus, breeding programs should aim at varieties exploiting additive gene action and heterotic combinations.

4.6.3 Salinity and Aluminium Tolerance

Plants subjected to salt stress exhibit perturbations in plant growth due to impaired nutrient and water uptake, oxidative stress and many more. A comprehensive review on impact of salt tolerance on grain legumes is presented in Farooq et al. (2017). Despite several strategies in evading off the salt stress, breeding is a cost-effective way in combating salinity stress in cowpea. Past efforts have succeeded in developing salinity tolerant soybean varieties (Patham and Lee 2007) though cowpea verities tolerant to salinity stress are less available. Win and Oo (2015) used salt tolerance index to classify Cowpea genotypes, which fell into twodistinct classes of tolerant and susceptible. These results apparently indicate salt tolerance to be governed by qualitative loci which was confirmed by association analysis (Ravelombola et al. 2018). Nevertheless, salt tolerance can only be measured indirectly through agronomic traits under salt stress, hence salinity tolerance should be a quantitative measure.

Aluminium stress while widely prevalent on Oxisol and Ultisol soils around the tropics and semi-tropical regions (Marschner 1991)has not been well studied in relationship to tolerance traits in cowpea (DeManzi and Cartwright 1984), as this crop was considered well adapted to these environments given its heat tolerance. In a recent study, Ajayi (2021) determined genotypic differences in aluminium tolerance of cowpea accessions utilizing germination parameters but adult plants can react differently to the toxicity than seedlings. Hydroponics can be used with slightly older plants but is usually applied to legume prior to flowering (Blair et al. 2009). Sources of greater tolerance would be useful as cowpea expands to regions that could be made productive by liming such as the Cerrados of Brazil and central Africa (Bojórquez-Quintal et al. 2017).

4.6.4 Herbicide Tolerance

In the era of mechanization, herbicide usage has eased the crop production nevertheless demanding varieties that tolerate commercial dosages of herbicides. Cowpea being a smothering crop, demands no weeding operations. However, crop demands weedicide interventions for potential yield realization. Herbicide tolerance, in this context, is of prime value in mechanizing farming. Natural tolerance to sulfentrazone was reported in the cowpea genotypes studied by Ikeda et al. (2021). Differential expression of Cowpea genotypes to Bentazon application was reported by Harrison and Ferry (1993). Similarly, Burgos et al. (2007) reported variable response of advanced breeding lines of Cowpea to Fomesafen application. However, transgenic breeding has transferred bar genes to cowpea varieties rendering them tolerant to imazapyr (Citadin et al. 2013) and Gluphosinate (Aasim et al. 2013).

4.7 Needs in Breeding and Genetics of Cowpea

The United Nations Department of Economic and Social Affairs predicts the world's population to reach 9.8 billion people by 2050 (United Nations 2019). With dwindling water supplies in many areas of the globe and demand for arable land increasing steadily, it is without question that agricultural practices focused on vigor agronomic productivity and resistance are required for the world's future food security. Cowpeais a crop that is widely grown in areas that are already having issues with food scarcity, including sub—Saharan Africa (Huynh 2013). This makes research and additional progress in cowpea improvement a good way to address the increased demand for agricultural food products.

Cowpea is a climate-resilient legume crop grown in hot and dry climate regions of the world (Hall 2012). About 10 million hectares were grown about a decade ago and five million tons of cowpea grain harvested each year globally (Andargie et al. 2013) with this number increasing as greater need for inexpensive protein source is required. It is grown by small farmers for its multipurpose use like food, fodder, and vegetable. Cowpea productivity is affected by various biotic and abiotic stresses. Systematic breeding efforts from the last 3 decades have been led by conventional breeding, and the progress is very limited.

Narrow genetic base and lack of focused conservation of plant genetic resources of endangered and wild relatives, and slow pre-breeding pace have to be strengthened to break yield plateau. Trait discovery in specific germplasm and multi-location evaluation of traits is required to make them available to be used in breeding programs. Multiple stress-tolerant cowpeas with traits conferring tolerance to drought, heat, aluminium or manganese stresses with high nitrogen fixation and phosphorous use efficiency are yet other important aspects that need attention since cowpea is grown by small farmers in marginalized land with less input. Simultaneously efforts should also be made on improving nutritional qualities of the crop, with high protein content,

Table 4.5 QTL numbers, authors and publication journals for abiotic stresses and related plant phenotypic traits

Trait	Reference authors	Number QTL	Journal
Heat tolerance	Lucas et al. (2013)	5	Molecular breeding
Drought tolerance	Muchero et al. (2009)	12	TheorAppl genetics
	Muchero et al. (2011)	9	BMC genomics
Salinity tolerance	Fan et al. (2015)	3	BMC genomics
Root characteristics	Burridge et al. (2020)	11	TheorAppl genetics
Ineffective nodulation	Ohlson et al. (2018)	2	Crop breeding and genetics

high fiber, and low glycemic index with high levels of antioxidants to eradicate the threat of malnutrition and non-communicable diseases in Asia and Africa. Pulses, including cowpea, are high in protein compared to most other crops.

QTL studies are directly needed for identifying genes and germplasm for these quality traits and abiotic stress resistances. Two general methods are used currently for QTL detection; namely, cross-derived population studies whether these be bi-parental or multi-parent in origin and naturally derived populations of accessions. In the latter case a statistical procedure called genome wide association (GWAS) studies identify markers, usually SNPs that are associated with quantitative or semi-quantitative phenotypic traits. However, few QTL studies have been conducted for abiotic stresses and small number of QTL loci overall in cowpea (Table 4.5).

The resistance to abiotic stress discussed in this chapter shows that cowpea is already a crop with widespread agricultural use and one that could benefit greatly from more research into improving its ability to provide food and its yield limitations. Currently there are a lot of different focuses on cowpea research, for example improving seed size (Egbadzor 2013) or understanding flowering time (Andargie et al. 2013). While sources of salinity and drought tolerance are well documented (Hall 2012) aluminium and manganese toxicity tolerance and adaptation to nutrient deficiencies such as low phosphorus are less well studiedthan easy to measure phenotype. The genetics of these abiotic genes and QTL are not well understood even though these stresses are widespread in the world for most plants (Delhaize and Ryan1995) and for cowpea in particular Horst et al. 2000; Kolawole et al. 2000). Sources for better biological nitrogen fixation also need to be investigated.

The use of molecular tools for DNA fingerprinting of superior accessions/germplasm will help in breeding programs. Conventional breeding efforts and biotechnological advances should go hand in hand and should always complement each other for developing multiple stress-tolerant, climate-smart cowpea varieties for different ecological niches to address food and nutritional security in developing countries and to meet sustainable development goals. Various reviews have discussed the importance of molecular marker tools development in cowpea so will not be covered here, although their relevance to abiotic stress breeding is undeniable.

4.8 Future Prospects in Genetic Improvement

Molecular breeding has been the foundation for 21st century crop improvement. In particular, marker-assisted selection (MAS) has been successfully used in selection of specific major genes/alleles in plant breeding (Collard et al. 2005). Current availability of high throughput sequencing has increased the capability for MAS exponentially although costs are still high. For example, an Illumine based consortia for cowpea has put over 50,000 SNPs on a genet chip that can be used in screening or selection of germplasm. SNP technology is the molecular-marker platform of choice in genome-wide mapping, association studies, diversity analysis, and tagging of important genes in plant genomics and breeding. However, databasing of the GWAS and QTL results is woefully under-sourced for cowpeas and indeed for most legumes, althoughSNP maps for cowpea have been constructed (Muchero et al. 2009) and the physical map of the cowpea genome is available on the phytozome website (Lonardi et al. 2019).

The genome is 519.4 Mb in size and organized into 11 chromosomes and 722 scaffolds. In that study, 29,773 genes were annotated based on 42,287 transcripts. The selection based on suites of genes would be useful as they become associated with QTL and genes for abiotic stress tolerance. Conducting mega-analyses of studies on abiotic stress tolerance and leveraging of cross legume information could be done through more extensive database curation (Bauch et al. 2019). Mutation would also be useful to uncover the function of genes annotated by sequencing but whose purpose is not well studies. Mutant drought tolerant lines were found by de Ronde and Spreeth (2007) but mutation studies have not been conducted for other traits.

A new method of directed mutagenesis and gene function analysis involves genetic editing of specific DNA sequences using the CRISPR system and similar bacterial-derived enzymes such as *Cas9*/*Cas13*. These can target specific genes through guide RNA based sequences (gRNA) homologous to a gene sequence in the genome of cowpea or its upstream/downstream promoter/enhancer segments. The result of gRNA mutation is to change the DNA sequence or expressionof gene of interest in a given variety to replicate a desired allele or create a completely new single nucleotide polymorphism that changes expression of a gene. This allelic modification holds great promise for legumes and its potential uses have been summarized recently for the Legume family (Bhowmik et al. 2021) although few released CRISPR lines have been gene edited so far in pulses.

More studies are needed to analyze specific candidate genes involved in abiotic stress to be able to fulfill the promise of gene editing technologies. For example, Fecht-Christoffers et al. (2003) evaluated apoplastic peroxidases and ascorbate involvement in manganese toxicity tolerance with implications in rhizosphericorganic acid exudation and organic matter accumulation in controlling aluminium stress as well (Berggren and Mulder 1995). Candidate genes for drought tolerance have been better studied in plants overall but in cowpea only a few have been proposed such as those by Muchero et al. (2010). These authors proposed multidrug resistance

and photosystem I assembly proteins as underlying QTL for adaptation to drought in the crop.

The drought responsive gene found to be important in many model plants and crop species, DREB2 is certainly a candidate gene for drought tolerance in cowpea based on the transgenic testing by Sadhukhan et al. (2014). Reference genes were found for salt and dehydration stress on roots for gene expression studies using qPCR (Amorim et al. 2018) but equally showed the pathways important to resist these abiotic stresses, such as the flavonoid genes, chalcone isomerase and chalcone synthase, which were up-regulated.

QTL analysesare often useful for determining the importance of candidate genes in real life situations of abiotic stress and agricultural production systems. As discussed very few QTL studies have been completed in cowpea, compared to other crops so the number of QTL loci annotated is small. Future breeding efforts should expand the database of abiotic stress responsive genes while simultaneously developing germplasm useful for crop improvement programs.

The importance of cowpeas to present and future food security is undeniable. Cowpea ranks as the fifth highest source of plant protein for human consumption globally and with 17–30% dry weight matter beingprotein, it is twice that of cereals (Jayathilake et al. 2018). Combined with their nutritional value, cowpeas are suited for areas of water scarcity and marginal soils. Agriculture is highly dependent on soil and weather and areas with marginal soils are the most affected by low food production and general loss of resources. Regions of greatest food insecurity combine dense population, susceptibility to climate change, and dependence on the agricultural sector including cowpeas (Boukar et al. 2018). This combination of factors makes areas much more vulnerable with respect to food availability, income generation and overall livelihood.

Areas of food insecurity and marginal soil are found in large parts of Sub Saharan Africa and in South and Southeast Asia. These regions have had numerous struggles with food insecurity and weather-related agricultural hardships. The future of climate change predicts worsening trends for these areas. In South Asia, there is high susceptibility to future climate change (Aryal et al. 2020). South Asian climate is set to see an increase in mean temperature above the global average. In models of crop response to climate change, the potential output for crops lowers and becomes unstable because of the need for substantial water use to combat the rise in temperature. Additional factors contributing to the potential detrimental effects of climate change can be found all over the world but in several regions of the world the heat and drought tolerant cowpea will be called upon to have a high impact on future agriculture.

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Chapter 5
Breeding for Abiotic Stress Tolerance in Lentil in Genomic Era

Akanksha Singh, H. K. Dikshit, G. P. Mishra, M. Aski, Shiv Kumar, and A. Sarker

Abstract Lentil (*Lens culinaris* subsp. *culinaris*) is a self-pollinated cool season food legume crop and it ranks fifth in global production of pulses. Lentils have an excellent nutritional profile and are easily digestible pulse crop. Global climate change lead to high incidence of abiotic and biotic stresses that impeded the production and productivity of lentil. The major abiotic stresses impacting lentil are salinity, waterlogging, cold, drought and heat that limits the crop yield and to resolve this it is important to develop climate resilient lentil varieties. In this chapter, we discussed the impact of several abiotic stresses on lentil production, genetics, genomics including mapping of quantitative traits and incorporating the identified genes with the assistance of marker assisted breeding and transcriptomics for development of abiotic stress tolerance in lentil. To achieve the goal of developing tolerant varieties utilization of the genetic resources through screening, selection and introgression is the key of any breeding program. The advance genomic technologies can complement conventional breeding approaches for acceleration of breeding programs by increasing the precision and reducing the time through identification of candidate genes, gene mapping, marker assisted selection. Precise and repeatable phenotypic screening techniques are essentially required to screen the germplasm and breeding material which help in developing cultivars tolerant to abiotic stresses. Limited reports are available on tolerance to abiotic stresses in lentil and further investigations is required to understand the underlying genetic mechanism.

Keywords Abiotic stress · Lentil · Tolerance · Conventional · Molecular breeding

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5.1 Introduction

Lentil (*Lens culinaris* subsp. *culinaris*) is a self-pollinated grain legume grown in winter season. The ancestral origin of the crop is Eastern Mediterranean region (Gupta et al. 2011). Lentil is mainly cultivated in the Mediterranean regions, south-west Asia and further it has spanned its horizon with introduction in Australia, South and North America (Hamwieh et al. 2009). Lentil grains are abundant in proteins, folate, vitamin C, fiber (Bari et al. 2009), carbohydrates and minerals. Lentil grains are superior as compared to other grain legumes and cereals due to high total phenolic content (Xu et al. 2010) as well as low phytic acid content (Thavarajah et al. 2011). Lentil grains are excellent source of nutrients, amino acids and good quality protein (Khazaei et al. 2016) and are useful for maintaining the overall health of human beings as well as animals (Kumar et al. 2015; Kumar et al. 2019). Lentil consumption decreases body fat and body weight (Siva et al. 2018) and maintains antihypertensive function (García-Mora et al. 2017).

The crop has been cultivated in around 58 countries, and the highest production has been recorded in Canada, contributing approximately 48.1% of the global lentil production and also being the top exporter of lentil around the world. India ranks second in the global production but is the largest importer of lentil (Dissanayake et al. 2020). Moreover, the global average yield of lentil is about 926 kg/ha while the average yield of Asia is 817 kg/ha, which is considerably lower than the global productivity (Singh et al. 2017). Irrespective of its importance for animal feed, human food, and cropping system, the crop remained underutilized. Lentil varieties, under cultivation have been developed by hybridization with the traditional lentil varieties for improving disease resistance along with improving yield. Despite that, there has been very little progress for improving the production and productivity of this crop. Khazaei et al. (2016) reported narrow genetic base of lentils grown in Canada and South Asia making it susceptible to abiotic and biotic stress environment. The narrow genetic base of cultivated lentil germplasm can be broadened by hybridization with related wild species (Duran et al. 2004). Only recently the modern genomic techniques are being utilized to breed climate resilient lentil varieties.

5.1.1 Nutritional Value of Lentil

Lentil is one of the most vital foods known from the prehistoric period (Sarker and Erskine 2006). Its dietary significance is recognized for fulfilling the food and feed requirement of human beings and animals. Distinct recipes of lentil have been prepared and consumed in South Asia. In the Indian subcontinent it has been consumed as “dhal”. In Mediterranean regions “Mejadra” is prepared which is the combination of lentil and rice and is also famous with its name as ‘mujaddra’. The flour of lentil is utilized for preparation of puree, soups, stew and can be mixed with cereal flour for preparation of cake and bread. Due to short cooking time, loss of

nutrients during cooking is less in lentil. Lentils have an excellent nutritional profile and are easily digestible pulse crop. Lentil grains are called as "poor man's meat", due to the richness in the nutritional content, 20–36% protein, 60–67% carbohydrate, 2–3% ash on a dry basis and <4% lipid (Bhatty 1988). Lentil have a similar nutritional profile as other food crops such as chickpea, soybean, rice and wheat (Johnson et al. 2020). It is considered as the most suitable source of protein in comparison to soybean and chickpea due the negligible content of antinutrients, fat, cholesterol (Sultana and Ghafoor 2008) and low lipids, and in comparison to rice and wheat it have superior minerals and vitamins profile (Johnson et al. 2020). Lentils are deficient in cysteine and methionine (sulfur-containing amino acids) as well as in tryptophan.

As a consequence diets comprising of legumes including lentils serve several health benefits. As legumes, specially lentils have a lower glycemic index. It lowers the risk of diabetes (Becerra-Tomás et al. 2018). The glycemic index of diet based on red lentils are very low (21%) in comparison to cereal based diets such as basmati rice (69%), multigrain bread (62%) and whole-wheat pasta (55%) (Henry et al. 2005). A regulat diet including lentils helps in reducing the risk of cardiovascular diseases by reducing lipoprotein cholesterol (Abeysekara et al. 2012, 2010), and also facilitate in combating obesity (Siva et al. 2018). Lentil possesses prebiotic carbohydrates having several heath benefits (Johnson et al. 2013). Rizkalla et al. (2004), reported that lentils can be utilized for treating type II diabetes.

Sharp decline in the fasting blood glucose has been reported due to consumption of 50 g of lentil in the diet of diabetic patient's (Shams et al. 2008). There are multiple studies which reported that consumption of lentils results in overall health of human beings by enhancing serum antioxidant capacity, alleviating oxidative stress, regulating the progress of cardiovascular diseases, reducing adhesion molecules and inflammatory biomarkers and triglycerides, (Azadbakht et al. 2007; Crujeiras et al. 2007; Esmaillzadeh and Azadbakht 2012).

5.1.2 Reduction in Yield and Quality Due to Stress

The production and productivity of pulse crop has been impeded due to environmental stresses caused by biotic and abiotic factors. Furthermore lentil is more susceptible to these factors due there narrow genetic base in comparison to their crop wild relatives (Singh et al. 2013). The yield of lentil is reduced due to exposure of crop to various stresses (Muehlbauer et al. 2006).

The major abiotic stresses are drought, temperature (high and low), salinity, waterlogging, deficiency and toxicity of nutrients (Yau and Erskine 2000). Among these cold, salinity, heat and drought stresses are of global concern (Silim et al. 1993; Turner et al. 2001) (Fig. 5.1). The lentils grown in South Asia are of *Pilosae* type with narrow genetic base. The low variability in phenological, morphological and yield contributing traits and vulnerability to the major abiotic stresses have slowed down the progress in lentil improvement. The conventional breeding methods have successfully developed varieties resistant to major abiotic stresses (Sarker and Erskine 2006;

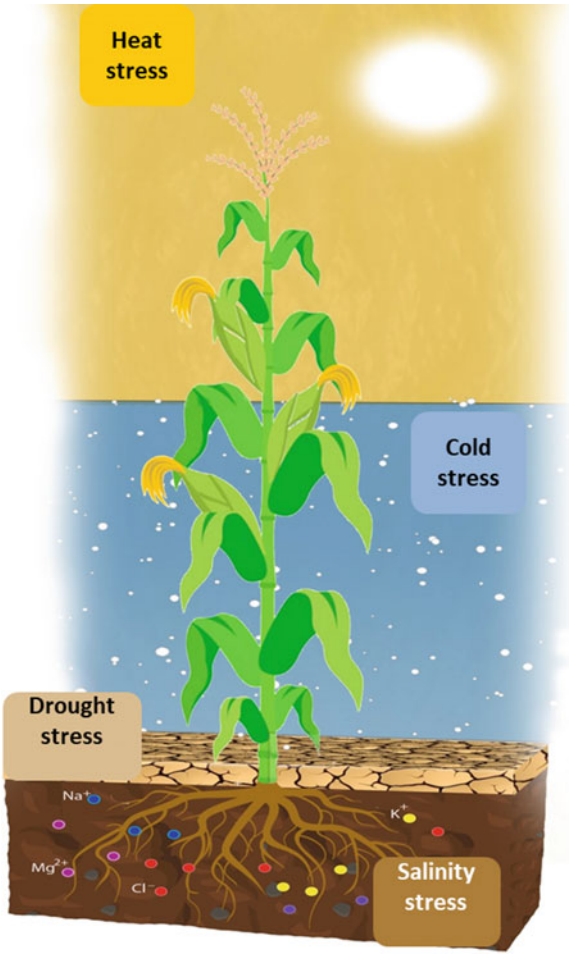

Fig. 5.1 Major abiotic stresses affecting legumes

Muehlbauer et al. 2006; Materne and McNeil 2007). Nevertheless, breeding programs have been facing limitation in terms of narrow genetic base, absence of precise selection methods, and non-availability of genetic information which hamper the overall breeding progress.

5.1.3 Morphological Traits for Improving Productivity

In lentil breeding, morphological traits hold great importance. One of the most vital traits of lentil is day to flowering which is the key trait for acclimatization and adaptation of lentil genotypes in a new geographical region (Wallace et al. 1993). In North America and in South Asian countries breeding program focuses on developing

short season lentil varieties to provide stable and improved production (Tullu et al. 2008).

The timing of the flowering in lentil is significantly influenced by temperature and photoperiod in which crop is grown (Sarker et al. 1999). In order to target and expand the cultivation of lentils in South Asia region the focus should be development of early maturing varieties which can withstand terminal heat and short day conditions (Erskine et al. 1998). Total biomass is an important trait in West Asia and North African regions for lentil production as specifically in this region the harvest of the crop is utilized for the comsumption of human beings and straw is used as fodder to feed animals (Silim et al. 1989). Positive correlation has been identified for seed and straw production and simultaneous selection can be done for both the traits (Hamdi et al. 1991; Tullu et al. 2001). Furthermore exploring the variation within germplasm assist in tapping the wide genetic variability and thereby identified genes can be introgressed into cultivated gene pool to broaden the genetic base (Singh et al. 2011).

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

The climate change affects different aspects of agricultural systems resulting in food insecurity and total crop failures globally. Legume crops already deal with several stresses, however change in the climatic conditions make it complicated to cope up with this environmental changes. Simulation reports and research on climate change predict that by late 21st century the temperature will rise by 2–4 °C (Girvetz et al. 2017) and this change in temperature directly effects the production and productivity of the crop including legumes (Varshney et al. 2011; Zhao et al. 2017). Identification and introgression of variable traits or genes responsible for broadening genetic base of the cultivated genotypes is vital for development of climate resilient lentil genotypes. This chapter focuses on an insight of the abiotic stresses of lentil including screening protocols, identification of tolerant sources, biochemical mechanism involved, and the role of conventional breeding, molecular tools, genomics for identification and utilization of stress tolerant genetic resources.

5.2 Abiotic Stresses Affecting Lentil

5.2.1 Heat Tolerance

High temperature stress hinders different growth stages during crop developmentin lentil. This crop is sensitive to the rising temperature as other cool season grain legumes (Sehgal et al. 2017; Choukri et al. 2020). Different stages of the growth

of the plant require different temperature which ranges from 18–30 °C. During the vegetative growth stages it requires cooler temperatures while warmer temperatures are required at the time of maturity (Choudhury et al. 2012). The crop has been grown in comparatively warmer regions of India (South and North) and exposed to supra-optimal temperatures which results in reduction of crop yield (Verma et al. 2014). However it has been recorded that due to the climate change heat period has been longer in comparison to the chilling period which results in exposing crops to higher temperature stress and further reducing the yield of the crop (Hasanuzzaman et al. 2013). As reported by Delahunty et al. (2015), heat wave (35 °C) for continuous 6 days results in declining 70% of lentil yield across southeastern Australia. The maximum temperature above 32/20 °C (the ratio represents maximum and minimum temperature) at reproductive stage in lentil can sharply deteriorate the quality of the grain and also reduces the grain yield (Delahunty et al. 2015). The most sensitive stage is the seed filling stage, which is affected by heat stress, as a result grain yield are low and quality of the grain is also poor (Tickoo et al. 2005).

In lentil, temperatures above 24.4 °C reduce the rate of germination (Covell et al. 1986). The consequence of heat stress is decline in percentage of germination, abnormal growth of seedlings, degeneration of nodules, loss in cell membrane stability, reduction in plant biomass, early flowering, increase in lipid peroxidation and decrease in photosynthetic efficiency (Jiang and Huang, 2001a, b; Sehgal et al. 2017). The photosynthesis occurring in leaves was identified as the most sensitive stage and the mechanism gets impeded due to reduced rate of assimilation and carbon fixation, electron flow imbalance, chlorosis in plant, thermolability of photosystem II (Sinsawat et al. 2004). In heat tolerance lentil. Chakraborty and Pradhan (2010) reported enhanced expression of ascorbate peroxidase (APX). In lentil, tolerance to heat stress is responsible for increased level of antioxidants in leaf and superior pollen function (Sita et al. 2017). Few reactions of the plants subjected to heat stress environment are pollen sterility, flower drop, reduced seed set, pod abortion, shortened reproductive period, and forced maturity (Bhandari et al. 2016). In lentil, limited studies has been performed for screening of lentil genotypes for heat tolerance, although genetic variability have been detected in a few studies as reported by Gupta et al. (2019).

Lentil genotypes can withstand critical temperature of > 35/25 °C to survive/reproduce (Kumar et al. 2019). Hence for identification of resistant genotypes various methods have been developed for screening genotypes in field conditions and in controlled environment such as hydroponics, pot assays and pot experiments (Singh et al. 2017).

To screen the lentil genotypes for heat stress, they are grown in late sown conditions so that the temperature at reproductive stage is above 35 °C and the data of grain yield, pod/seed set can be collected and utilized for identifying genotypes that are tolerant to heat stress (Kumar et al. 2016; Sita et al. 2017; Singh et al. 2019). Nevertheless the flowering time of the genotypes differs due to variation in temperature under field conditions during reproductive stage (Kumar et al. 2016) and therefore causes hindrance in determining early flowering heat tolerant genotypes. It becomes

extremely difficult to evaluate for heat tolerance with precision under field conditions, therefore advancement in the phenotyping profiling techniques (phenomics) can be utilized for evaluating heat tolerant traits in the field (Basu et al. 2015; Mir et al. 2019). The cultivated genotypes of lentil have been evaluated in field grown conditions for pollen viability, photosynthetic rate and membrane stability traits with high precision laboratory techniques to identify heat tolerant and susceptible genotypes (Kumar et al. 2018). Under extreme heat conditions germinating seeds have been evaluated in controlled conditions for identification of heat tolerant genotypes (Roy et al. 2011; Singh et al. 2016). Nevertheless to identify heat tolerant genotypes it is better to evaluate them under field and controlled condition as solely evaluating under controlled condition would do not reproduce the results under field conditions. Singh et al. (2017) evaluated lentil genotypes from the first day of anthesis by exposing them to continuous heat stress environment (35/20 °C) for 4 h consecutively for 7 days under controlled conditions which is followed by prolonged exposure to normal temperature (27/16 °C) for the development of pods. In some other studies conducted by researchers, plants were evaluated in pots in field conditions and later on shifted into controlled conditions at the flower initiation stage to expose them to suitable temperature during anthesis (Sita et al. 2017; Chen et al. 2019). Lentil accessions grown in high temperature regions to identify genes tolerant to heat stress which can be further utilized in screening, identification and introgression in the cultivated genotypes (Delahunty et al. 2015; Gaur et al. 2015).

5.2.2 Cold Tolerance

Lentils exhibit sensitivity to frost, the level of sensitivity of lentils can be compared with other legumes for example it is more sensitive than chickpea but it is less susceptible than pea (Murray et al. 1988). In the early stage of growth, plants recover instantly from underground axillary buds. Despite this, at maturity stage or after that if exposed to frost, plant die as the initiation of axillary buds ceased and further restricts the plant to go further to reproductive stage. Maximum injury due to frost occurs at flowering stage due to exposure of flowers to frost leading to smaller size of pods. In lentil, symptoms of frost injury are abortion of flower and pod, injury to the vegetative tissue (Gupta et al. 2019). During the development of pods and at the time of pod filling, seed coat can be damaged by frost and further frost affects the overall development of seed. Stem wilting and leaf damage have been observed in severe frost conditions. Due to the continuous exposure to frost it makes the plant vulnerable for entry of pathogen and results in susceptibility to diseases such as Botrytis gray mold and anthracnose (Gupta et al. 2019) and results in severe yield reduction. Breeding efforts have been made for characterizing frost tolerance in lentil by various researchers (Summerfield et al. 1985; Murray et al. 1988; Spaeth and Muehlbauer 1991; Ali et al. 1999). In some of the recent studies frost injury and winter hardiness have been studied in lentil (Kahraman et al. 2004; Barrios et al. 2007, 2010, 2016).

5.2.3 Drought Tolerance

Among all the legumes lentil exhibits moderate tolerance to drought stress (Reda 2015). Lentil can be grown in dry regions as it requires minimal water for its growth and development but under drought stress environment its yield is reduced by 6 to 70% and in severe cases drought leads to total crop failure (Babayeva et al. 2014). The production of lentil is altered by variable patterns of rainfall which leads to more frequent drought periods (Dai 2011). At different stages of development drought effects are variable; 70% and 24% losses were recorded at pod development and reproductive stage, respectively (Shrestha et al. 2006; Allahmoradi et al. 2013) and drought further results in reducing the leaf area. Drought stress at the flowering or the pod development stage impacts vegetative phase and also severely impacts reproductive phases leading to reduced leaf area, flower production, number of pods and seeds, total dry matter with significantly higher abortion of pods and flower drop (Shrestha et al. 2006). Drought stress also affect metabolism, osmoregulation and concentration of photosynthetic pigments in lentil (Gokcay 2012; Muscolo et al. 2014; Mishra et al. 2016; Biju et al. 2017). Changes in the pattern of annual rainfall further escalates the frequency of drought periods during the cropping season and therefore threaten the sustainability of lentil production (Dai 2011). The crop productivity depends upon the soil moisture in case of lentil as 90% of the cultivable area in lentil is the region where growth of the crop is dependent upon conserved and receding soil moisture and (Kumar and Van Rheenen 2000).

Drought avoidance and tolerance are the two mechanisms involved in lentil for combating drought stress. There are different mechanism to support drought tolerance in lentil which includes regulation of opening and closing of stomata, adjusting the osmotic pressure, enhanced antioxidant responses, dense pubescence of leaf, and increase in the yield components. Early maturing varieties exhibit drought-avoidance mechanism for example Idlib 3, Precoz, Bakaria, Bari Masoor 4, Bari Masoor 5 and Bari Masoor 6 (Erskine and Saxena 1993; Erskine et al. 1994; Shrestha et al. 2005). Studies by Singh et al. (2014) and Pratap et al. (2014) exhibited the association of agro-morphological traits with drought tolerance.

Traits associated with shoot such as the length of the stem, surface area of leaf, structure canopy, movement of leaf, stomata related traits have substantial role in drought avoidance mechanism (Salam and Islam 1994). Root traits are important components of drought avoidance and can be exploited for development of genotypes with drought avoidance mechanism in lentil breeding (Idrissi et al. 2016; Biju et al. 2017). Therefore selecting root related traits gives immense opportunity to improve the grain yield under the optimum nutrient and water conditions and also in poor soil resources (Gahoonia et al. 2005; Chen et al. 2015). Different mechanisms prevail for above-ground lentil for protecting plant exposed to water deficient conditions for example traits such as early or delayed flowering and pubescence. In wild lentils, mechanism of drought escape is inconsiderable in comparison to cultivated lentils (Hamdi and Erskine 1996). In a recent study by Gorim and Vandenberg (2017a) wild lentil accessions have been screened for variable drought mechanisms by analyzing

root related traits and revealed that diverse strategies have been employed in wild lentils such as reduced plant height, delay in flowering, and reduction in the transpiration rates. Although research has been carried out but still underlying mechanism involved for coping the wild lentils against variable annual rainfall environment is still not clear. Breeding programs has been targeted towards the two strategies: short term strategy for assessing the genetic variability for drought tolerance in lentil germplasm and long term strategy to introgress desirable traits from wild species to the cultivated one. These breeding strategies permit the production to be sustained during drought stress environment (Erskine et al. 2011). Initially osmotic adjustment (increase in solute concentration in cell) for maintaining turgor at low water potential (Kumar and Elston 1992) was regarded as an adaptation to drought. There have been different reports on the role of osmotic adjustment in turgor maintenance and its relationship with yield of crops (Morgan and Condon 1986; Passioura 1981; Munns 2002) were published. Drought stress tolerance can also be evaluated by estimating variables such as relative water content (Schonfeld et al. 1988), stomatal conductance (Terzi et al. 2010), water use efficiency and seedling vigor of genotypes subjected to drought stress (Nagarajan and Rane 2000; Dhanda et al. 2004). Kumar and Singh (1998) and Hura et al. (2009) have reported drought screening methods based on morphological, physiological and biochemical traits under soil condition. In lentil a rapid screening method was reported by Singh et al. (2013). A few of the early researchers suggested characterization of root and shoot attributes at seedling development and plant growth stages by dehydration avoidance (Kumar et al. 2012). Root attributes such as morphological and physiological parameters related to depth, length and density, shoot traits like rapid ground cover, early growth vigor and leaf characteristics are related with drought tolerance and assist in assessing transpiration demand of plant (Passioura 1981). Traits such as long and highly branched roots improve the capacity of absorption of water and nutrient from the depth of soil (Gahoonia et al. 2005, 2006). There is a variation in the type of root requirement according to the crop and species. Nevertheless study on legume root system is very less and in lentil limited information is available on the root and shoot systems (Gahoonia et al. 2005, 2006; Sarker et al. 2005).

5.2.4 Flooding and Submergence Tolerance

Globally, flood is responsible for the two-third of crop production losses in between the year 2006 to 2016 (FAO 2017). Submergence and flooding drastically affect the yield of legume crops (Solaiman et al. 2007; Kang et al. 2017). Waterlogging hinders the lentil production in poor drainage soil such as duplex types, subsoil compaction, fine textured or in conditions of persistent of extreme rainfall (Wiraguna et al. 2017). The damage occurred due to waterlogging vary with duration of the stress, its severity and growth stages resulting in absolute loss of crop in the severely affected conditions (Toker et al. 2011). Among the legumes, lentil is most sensitive to waterlogging

(Solaiman et al. 2007) and transient waterlogging is a major impediment for production of lentil, specifically in early vegetative growth stages. (Materne and Siddique 2009). Waterlogging affects all the development stages in lentil and subsequently reduces the yield (Materne and Siddique 2009). At germination stage, it results in delay in seed germination, and further suppression in the root growth and development (Jayasundara et al. 1997) and flowering stage is referred as the most sensitive stage to waterlogging causing abortion of flower and pods. Plant displays variety of symptoms including reduced growth, leaf withering, and waterlogging ultimately causes death of the plant. Wiraguna et al. (2017) screened lentil germplasm against waterlogging tolerance and showed that genotypes of Bangaldesh origin are tolerant to waterlogging stress at germination stage.

Waterlogging-tolerant genotypes were identified by their high root porosity, early flowering and maturity, low biomass and higher stomatal conductivity (Malik et al. 2015; Erskine et al. 2016). Currently, the strategies developed for overcoming waterlogging are based upon the avoidance. A few management practices effectively utilized for decreasing the adverse effects of waterlogging in lentil involves drainage, seeding rate, sowing time, and paddock selection (Toker and Mutlu 2011). Breeding for increased tolerance through selection for more arenchyma or adventitious root growth have been suggested as a possible solution (Jayasundara et al. 1997; Materne and Siddique 2009). Crop primarily rice and Arabidopsis have been widely investigated for flooding stress. However, there is a urgent need for focused research for abiotic stress in legumes, especially lentil that warrant additional investigation.

5.2.5 Salinity Tolerance

Salinity is deleterious to legume crops as it affects the biological nitrogen fixation and nodulation (Rai et al. 1985; Rai and Singh 1999) subsequently limiting the growth of root hair. Lentil roots are extremely sensitive to the salinity stress, restricting the rhizobium infection and root growth (Rai and Singh 1999; Van Hoorn et al. 2001). Plant under salinity stress had an impact on germination of seed, its growth and survival and subsequently the overall biomass (Akhtar et al. 2009; Mahmood et al. 2008; Ebbisa et al. 2015). Plants respond variably when exposed to the different growth stages Munns and Tester (2008) environmental conditions, relative humidity, soil–water status, available nutrients, and temperature (Lachaâl et al. 2002). The reproductive stage is the most vulnerable to salinity stress (Vadez et al. 2007) and germination stage is less sensitive than the early stages of vegetative growth (Al-Mutata 2003; Sakina et al. 2016). In lentil, salinity stress inhibits the growth by adversely affecting the plants' biochemical and the physiological mechanisms such as ion homeostasis (Hossain et al. 2017), photosynthesis, oxidative damage, membrane damage, γ-aminobutyric acid, osmolyte accumulation and proline metabolism (AL-Quraan et al. 2014; Hossain et al. 2017; Al-Quraan and Al-Omari 2017). Salinity also increases anthocyanin pigmentation in lentil leaves while decreasing flower and pod setting (Van Hoorn et al. 2001), and subsequently results in overall growth

and development of plant by reducing plant height, total biomass, biochemical and enzymatic activity and grain yield (Tewari and Singh 1991). Due to salinity stress the yield of lentil has been reported to be reduced by 90 to 100% (3 dS/m) and 20% (2 dS/m) at variable electrical conductivity (Golezani and Yengabad 2012). Screening of wild species for salinity stress exhibited that accessions of *L. culinaris* ssp. *orientalis* are the most tolerant to salinity (DAC-ICAR-ICARDA, Annual Progress Report 2014). Some other researcher also performed salinity tolerant experiments in lentil and identified salinity tolerant genotypes (Kumawat et al. 2017; Singh et al. 2017). Strategies need to be evolved to overcome the yield loss due to salinity. To reduce the salinity in the affected regions soil and water management strategies may be adopted, but amelioration processes are very expensive and more cost effective methods need to be identified. Hence, designing program for developing salt tolerant genotypes is to be considered as sustainable, cost effective and more precise, for enhancing and stabilizing yield of the crop in salinity affected areas.

Excessive salts in soil affect the growth of the lentil by disturbing water and nutritional balance of crop plants. Some of the studies suggested that lentil is one of the most salt sensitive legume crops (Ashraf and Waheed 1990). Singh et al. (2017) screened genotypes for salinity tolerance and found variation for the trait. It is utmost vital to develop effective screening method to develop salinity tolerant genotypes. In general, field and hydroponic screening methods are in practice for salinity stress. But due to lack of homogeneity in the environment and soil conditions it is difficult to perform screening in fields conditions. The difficulty arising due to screening in field conditions can be resolved by screening in hydroponic system. Traits such as reduction in seedling growth, reduced germination, visual salt injury, biomass accumulation, seedling survival, accumulation of proline, antioxidant activities, Na+, Cl−, K+ contents, hydrogen peroxide (H_2O_2) production have been studied by various researchers for evaluation of salinity tolerance in various crops (Singh et al. 2019). Studies reported that genotypes selection at seedling stage is a rapid and more accurate criterion for salinity stress tolerance (Gregorio 1997). Several biochemical and physiological traits like seedling growth and biomass, production of H_2O_2, seedling survivability, Na+ and Cl− accumulation, salinity score, antioxidant activities, etc. are evaluated for characterization for salinity tolerance at seedling stage in crop plants (Singh et al. 2017). The major traits which influence the salinity tolerance are FDA based fluorescent signals (indicating production of H_2O_2) and seedling survivability (Singh et al. 2017).

5.3 Lentil Wild Relatives as a Source of Tolerance to Abiotic Stress

The wild species of lentil are the pool of useful abiotic stress tolerance genes. Cold tolerance in *L. culinaris* ssp. *orientalis* (Hamdi et al. 1996) and drought tolerance in species *L. ervoides*, *L. odemensis* and *L. nigricans* have been observed (Hamdi

and Erskine 1996; Gupta and Sharma 2006). Crop wild relatives of India have been characterized for tolerance to drought and *L. nigricans* was identified to be the most drought tolerant species (Gupta and Sharma 2006). In a study conducted by Singh et al. (2017), hundred accessions of *L. culinaris* subsp. *Orientalis* have been tested on a hydroponic medium for salinity tolerance and many donors have been identified in lentil. In terms of root traits crop wild relatives in lentil showed substantial variation in root biomass, root distribution, and other root related traits (Gorim and Vandenberg 2017). A few genotypes of *L. odemensis* and *L. orientalis* exhibited deep root system and also showed delay in flowering and salinity tolerance although there was decrease in the overall pod numbers and yield. One *L. lamottei* accession, was noticed to have a high level of trichomes (tiny hair on leaves, stem and pods of a plant) and *L. tomentosus* exhibited reduced rate of transpiration (Gorim and Vandenberg 2017).

Recombinant inbred lines developed by hybridizing *L. odemensis*, *L. orientalis*, and *L. ervoides* were characterized and used for mapping of disease and drought tolerance traits (Sanderson et al. 2019; Omar et al. 2019). Limited research have been conducted for heat stress tolerance in lentil Sita et al. (2017). Singh et al. (2019) used genome-wide transcriptomic study and identified the heat tolerance gene in lentil cultivars. However, further research is required to understand the underlying mechanisms to elucidate heat tolerance. Different approaches can be exploited to get the tolerance or resistance genotype against abiotic stresses: (1) Field phenotyping of the accessions on the basis of climate history and (2) collection of the GPS data on the grounds of weather data and analyzing the curated data across frost, heat and drought stresses. The Focused Identification of Germplasm Strategy (FIGS) were utilized for detection of locations with extreme distributions for these stresses and also in identification of crop wild relative (CWR) accessions for stress tolerances (Street et al. 2008).

5.4 Genetic Diversity Studies in Lentil

The genetic variability in lentil has been explored with the help of various approaches (Poyraz 2016). DNA-based markers are used in several studies for characterizing diversified germplasm to expoloit the genetic variability in lentil (Fikiru et al. 2007; Lombardi et al. 2014; Wong et al. 2015; Idrissi et al. 2015; Yadav et al. 2016; Khazaei et al. 2016;). Among all the molecular markers available, single nucleotide polymorphism (SNPs) are the most abundant markers available within the genome (Agarwal et al. 2008). The SNPs can be detected within the genome by advance high-throughput sequencing technology (Kim et al. 2016). The platform based on next-generation sequencing (NGS) are reducing the cost sequencing, and genome by sequencing methods are now applied for sequencing the large and diverse genomes (Malmberg et al. 2018). Till date, the diversity studies based upon SNPs have been limited. Some of the researchers have characterized wild and cultivated global accession of lentil (Wong et al. 2015; Khazaei et al. 2016; Dissanayake et al. 2020).

5.5 Next Generation Technologies as a Platform for Genomics Aided Breeding

The narrow genetic base, large genome size, low density linkage map, nonavailability of candidate genes have restricted the progress in the genomics assisted breeding in lentil (Kumar et al. 2015). The advances in research in the GBS NGS technologies have laid the foundation for accelerating the development of molecular markers and therefore enhanced the genome sequencing project of lentil globally. Lentil genome assembly for variety CDC Redberry has been released by next generation DNA sequencing technologies (Bett et al. 2016). The construction of genomic map speeds up the discovery of QTLs/genes for the traits of economic interest. The comprehensive genetic map is still not available in lentil and more genomic resources are required for tagging genes for key economically important trait and to produce a consensus genetic linkage map. The importance of the field phenotyping has been also emphasized by Bett et al. (2016) for lentil germplasms at multiple locations for generation of closely linked molecular markers for traits of interest. The SNPs will be able to identify the mutations occurring due to chemical and the physical means and therefore can detect the mutant traits at molecular level. The sequence information can be utilized by reverse genetics approach for dissecting the trait function. Several innovations like RNAi technology, target-induced local lesions in genomes (TILLING), virus-induced gene silencing (VIGS), have led to understanding the underlying molecular mechanisms in lentil. These innovative approaches have led to enhancing the genomic resources for genetic improvement as well as utilization of the resources in lentil breeding programs. These advanced technologies allowed breeders to integrate marker assisted backcrossing and marker assisted selection in lentil breeding programs.

5.6 Transcriptome Analysis of Lentil in Response to Abiotic Stresses

There is limited research on stress responsive genes in non-model legumes such as lentil, mungbean, and pigeonpea exposed to the abiotic stress prone environments. In lentil, candidate genes have been discovered for drought tolerance (Singh et al. 2017). In lentil, transcriptome analysis offers useful resources, and can be utilized in the absence of complete genome sequencing which is lacking in lentil. Transcriptome sequence of the two genotypes PDL-2 and JL-3 exposed to drought stress were analyzed by utilizing Illumina HiSeq 2500 platform. Difference in the gene expression exhibited upregulation of electron transport chain, correct folding of protein, reduction of stomatal conductance, oxidation–reduction process, organ senescence and TCA cycle in drought tolerant genotypes in comparison to the the sensitive ones. While genes responsible for negative regulation of absicisic acid, GABA synthesis,

synthesis of cell wall protein, transcription binding etc. are downregulated in drought tolerant genotype in contrast to drought susceptible genotype.

NGS technologies have undergone tremendous advances in the last couple of decades, as a result of improvements it is now possible to identify novel genes associated with abiotic stresses (Varshney et al. 2009; Barrera-Figueroa et al. 2012). There are several reports of mining out novel genes through de novo sequencing correlated with abiotic stress in *Vicia faba*, and wheat (Fox et al. 2014; Arun-Chinnappa et al. 2015). Molecular mechanism underlying heat stress has been studied by Singh et al. (2019) by transcriptome analysis. Transcriptome analysis could reveal new genes, and pathways associated with mechanism involved in heat tolerance in lentil. Singh et al. (2019) performed de novo transcriptome analysis with two lentil genotypes JL-3 and PDL-2. Among both the genotypes JL-3 is sensitive and PDL-2 is tolerant to heat stress. Both the genotypes were exposed to variable heat stress conditions for identification of differentially expressed genes (DEGs) and their pathways. The tolerant genotype (PDL-2) revealed higher number of DEGs in contrast to sensitive genotype at 3d of continuous heat stress, including both upregulated as well as downregulated genes. It was identified that most of the DEGs were primarily restricted to secondary metabolic component and cell wall. Similar studies have been reported in many crops such as wheat, Arabidopsis etc. (Kotak et al. 2007; Qin et al. 2008). Gene expression in response to cold stress was investigated by Barrios et al. (2017) by utilizing RILs developed from hybridization of Precoz (cold tolerant) x WA8649041 (cold sensitive). RILs were examined Deep Super-SAGE transcriptome sequence analysis. The identified sequences coded for the proteins associated with proline, glycine, drought and cold regulated proteins, proteins associated with dormancy and other membrane proteins. These were generally but not exclusively overexpressed in the acclimated tolerant lines. Singh et al. (2021) carried out extensive transcriptomic profiling recently and differential gene expression was revealed for salt stress in lentil. The identified genes were found to be closely associated with the pathways involved in phytohormone-mediated signal transduction, nitrogen metabolism, cell signaling during stress, secondary metabolism, cellular redox homoeostasis.

5.7 Molecular Mapping of Tolerance Genes and QTLs

In lentil, four QTLs were detected for winter injury and five QTLs survival, respectively using a 106 RILs derived from the cross of WA8649090 x Precoz grown at multiple locations (Kahraman et al. 2004). Among all the QTLs identified, just single QTL was expressed in every location. Barrios et al. (2007) reported QTLs for frost tolerance under winter sown lentils and also identified that these QTLs are also related to yield. Further research exhibits that the QTL for yield and winter hardiness are associated closely within the same linkage group (Barrios et al. 2017). Differential expression for frost tolerance have been reported in a RIL population derived by crossing Precoz with WA8649041 by Super—SAGE transcriptome profiling (Barrios et al. 2010). Singh et al. (2016) identified single major gene *Sdt* for seedling survival

under drought in lentil (*L. culinaris* Medikus) in F_2 mapping population (JL-3 3 PDL-1). Eighteen QTLs were reported by Idrissi et al. (2015) to be associated with shoot and root traits related to drought tolerance for instance lateral root numbers, root length, root and shoot ratio and dry root biomass. In lentil biparental mapping population has been extensively used for identifying QTLs associated with several economically important traits. The first linkage map in lentil for drought stress tolerance was developed by Singh et al. (2017). The molecular markers identified in the various studies will help in introgression of desired genes in the cultivated varieties.

A linkage map, for yield and drought related traits identified 75 QTLs from RIL (L830 × Precoz) spanning with 291 simple sequence repeat (SSR) markers (Rana et al. 2016). Singh et al. (2017b) reported two major QTLs for heat tolerance in lentil. The identified QTLs have paved the way for identification of genetic markers linked to the phenotype and further dissecting the candidate genes for heat tolerance. QTLs for boron tolerance have been studied in the biparental mapping population developed from by hybridizing Cassab × ILL2024 (Kaur et al. 2014). In molecular breeding programs, development of linkage maps accelerates the process of abiotic stress tolerance breeding and subsequently attainment of goal can be achieved with precision and accuracy. The molecular markers identified assist in introgression of the gene of interest in elite genetic background. GBS and NGS technologies have aided in accelerating the transcriptome sequencing and genome sequencing projects and speeding up trait discovery and molecular mapping.

5.8 Marker-Assisted Selection (MAS) in Lentil

Marker assisted selection (MAS) accelerates the efficiency of the breeding programs, and breeders can utilize the approach for selecting genotypes with required combination of genes. This approach has certain limitation in lentil in contrast to major legumes, due to decelerate development in genomic resources in lentil (Kumar et al. 2014). The traits of economic importance affected by genotype and environmental factors and governed by polygenes therefore RILs and near isogenic lines are the best suited for precisely analyzing and dissecting the useful traits. Linkage analysis in lentil has been initiated by Zamir and Ladizinsky (1984) and the first ever linkage map of lentil based on DNA based markers was developed by Havey and Muehlbauer (1989). Generation of molecular markers based on PCR accelerated the studies on developing linkage maps in lentil. Morphological markers and molecular markers were utilized for the first time by Eujayl et al. (1998) from the mapping population developed by crossing *Lens* ssp. *culinaris* x *Lens* ssp. *orientalis*. The first intraspecific lentil map of lentil was reported by Rubeena and Taylor (2003) by incorporating resistance gene analog (RGA), 114 RAPD and ISSR molecular markers for identification of gene/QTLs.

The genomic library of lentil was constructed by Hamwieh et al. (2005) from lentil cultivar ILL5588 and genetic variability was studied with simple sequence repeats (SSR) markers (Hamwieh et al. 2009). Tanyolac et al. (2010) reported 11

linkage groups of lentil by employing ISSR, AFLP and RAPD markers. Additional genomic resources have been generated by Verma et al. (2014) for employment in lentil improvement programs. Biparental mapping populations have been employed in generating several linkage maps in lentil but due to less coverage and larger size of genome the information curated have limited practical application (Ates et al. 2018). A high-density consensus linkage map comprsing seven linkage groups has been developed using diversity arrays technology (DArT) which depicts seven chromosomes of the lentil genome (Ates et al. 2018).

Recent advancement in molecular tools and development of genomic resources has lead us to utilize multiple mapping population rather than a single mapping population to speed up the generation of consensus linkage maps. Chip based markers are more prevalent now in comparison to PCR based markers for NGS approaches. Researchers developed plenty of SNP markers, for construction of lentil linkage map (Sharpe et al. 2013; Temel et al. 2015). The advancement in genome sequencing of lentil led to discovery of candidate genes for multiple traits (Bett et al. 2016) and also led breeders to stimulate MAS in lentil breeding.

5.9 Conclusion

Lentil is extremely nutritional and stress tolerant crop and it is extremely essential for improving sustainability of food production systems under changing climatic conditions. There is immense opportunity in lentil crop improvement programs for further increase and stabilization of lentil productivity.

Advance genomic technologies plays a vital role in improving the breeding programs. The lentil breeding is highly benefitted by comparing few genotypes such as tolerant vs. sensitive for the analysis of differential response to a defined stress and also understanding signaling pathways, and underlying molecular mechanism related to abiotic stresses. According to the research strong and significant climate change has been observed globally and there is increase in the abiotic stress environmental conditions. Therefore future goal should be to breed climate resilient varieties which can withstand abiotic stress environmental conditions by combining conventional and advanced breeding technologies.

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Chapter 6
Genomic Design for Abiotic Stress Resistance in Pigeonpea

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Abstract Pigeonpea is a versatile food grain that grows in the regions of tropical and subtropical climates. Biotic and abiotic factors have a big impact on pigeonpea yields. This chapter gives an insight into the major abiotic stresses affecting pigeonpea production and productivity across the globe, such as drought, waterlogging (WL),

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salinity tolerance, temperature tolerance (heat and cold), photoperiod, and metal toxicity. These above-mentioned abiotic stresses are expected to exacerbate by changing climate scenario. Despite the fact that traditional breeding efforts paved the way for the use of genetic diversity in a large-scale, yield levels have not improved significantly. Abiotic stress screening among cultivars and germplasm through traditional methods is difficult as abiotic stress is influenced by soil and agronomic variables and is frequently complicated by significant variation in occurrence, duration, and intensity. High-throughput and innovative phenotyping platforms helps in achieving the precision and also speed up the assessment of pigeonpea germplasm for different abiotic stresses. Modern genomic, as well as phenomic technologies and also the latest speed breeding methods may greatly benefit and fast track the development of climate-smart pigeonpea cultivars. The recent advances in the in modern breeding technologies like quantitative trait locus (QTL) mapping, marker-assisted back cross breeding (MABC), marker-assisted recurrent selection (MARS), next-generation sequencing (NGS), genomic selection (GS), genomics-assisted breeding (GAB) and their application in the categorization of helpful traits related to abiotic stresses can alleviate and advance the process of trait transfer from wild and its relative species into the backgrounds of cultivated better genotypes. In this chapter, we summarise the options for combating abiotic stresses by exploring the recent genomic technologies based on trait-specific improvements as well as their potential limitations and challenges.

Keywords Abiotic stress · Molecular mapping · MAS · QTL · Genomic selection · Bioinformatics

6.1 Introduction

6.1.1 Economic Importance of the Crop

Pigeonpea (*Cajanus cajan* [L.] Millspaugh) is a multipurpose food legume that serves as a livelihood to resource poor farmers in tropics and subtropics. It has gone from being a neglected crop in previous years to a core commercial crop today. Pigeonpea is the 6th important crop in the global grain legume production following beans, peas, chickpeas, broad beans and lentils. It is the second most important grain legume in India after chickpea accounting an area of 5.83 m ha with a production of 4.92 million tons (Table 6.1) (Food and Agriculture Organization (FAO) 2018). To the global production, India contributes 72% followed by Myanmar (11%), Tanzania (7%) and Malawi (5%). Pigeonpea is nutritionally rich and per 100 g of dry seeds contains around 306 kcal of energy, 20.6 g protein, 1.8 g fat, 21.4 g dietary fiber and available

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Table 6.1 Pigeonpea scenario on a Global Scale (2018)

Country	Area	Production	Country	Productivity
India	55.83 (80)	42.9 (72)	Philippinm	1857
Myanmar	5.33 (8)	6.76 (11)	Malawi	1743
Tanzania	3.00 (4)	4.35 (7)	Myanmar	1270
Malawi	2.50 (4)	3.16 (5)	Burundi	1147
Kenya	1.37 (2)	0.88 (1)	Dom. Republic	1076
Haiti	0.89 (1)	0.86 (1)	Tanzania	1053
Others	0.62 (1)	0.46 (0.80)	Nepal	987
Uganda	0.40 (1)	0.25 (0.40)	India	768
World	69.93	59.61	World	852

Source FAO Statistics 2018 (Area-Lakh ha, Production-Lakh tonnes, Yield-kg/ha)
Note Figures in braces pertains to percent contribution to the world

carbohydrates of 41.0 g and micronutrients like iron (5.1 mg), magnesium (118 mg), phosphorus (242 mg), potassium (1530 mg), zinc (5.32 mg), copper (1.33 mg) and folate (340 μg) (FAO/INFOODS 2016).

In addition to its high nutritional value as a food crop, being used for multipurpose viz, animal feed, fuel wood, host for lac insect rearing, green manuring and it is more effective in soil conservation. The green seeds are cooked as a vegetable in Africa, Central America and the Indian states of Gujarat and Karnataka, while tender pods are cooked whole in Brazil, Thailand and the eastern islands of Indonesia, in addition to its principal use as dal (dry, dehulled, split seed used for cooking). Pigeon peas are produced for canning and consumption in select regions, including the Caribbean coasts of Colombia, the Dominican Republic, Panama, and Hawaii. Factors such as population expansion, higher living conditions and more consumer health awareness are now influencing the global market for pigeonpea (http://agrifact.weebly.com/red-gram-an-introduction.html).

6.1.2 Reduction in Yield and Quality Due to Abiotic Stresses

Despite the reality that India leads the world in terms of area and production; yields are low when compared to the respite of the world and some African countries. Farmers continue to grow their traditional landraces, which typically suffer from biotic and abiotic stress resulting in substantial decline in output. Other restrictions include low plant densities, inadequate soil fertility, poor weeding, and insufficient/inappropriate application of fungicides and herbicides. Productivity is also influenced by environmental and social (lack of roads, marketing infrastructure, and middleman exploitation) considerations. Weeds and various diseases are the major biotic stresses limiting pigeonpea productivity (Singh et al. 2020), but economic losses also occur more likely

due to abiotic stress imposed throughout its life cycle. Pigeonpea is subjected to an array of abiotic stresses, including moisture (WL/drought), temperature, photoperiod, mineral (salinity/acidity) stress and so on.

Pragmatic evidence shows that frequent interlink of one abiotic stress with several other stresses, making it difficult to understand the exact cause of crop failure. The literature information is poor on screening, tolerant genotypes identification and utilizing them to improve abiotic stresses in pigeonpea in comparison to cereals (Choudhary et al. 2011). Choudhary (2013) and Pooniya et al. (2015) found yield gaps in pulses ranging from 477 to 563 kg/ha at research field and farmer's fields. Drought and heat stress are two abiotic variables that can slash seed yields in half, especially in dry and semiarid regions. Chilling stress is most widespread in the tropical and subtropical regions of pigeonpea growing area and it has a considerable impact on productivity. According to Kumar et al. (2016), a temperature range of 0–10 °C is regarded as threshold for cold stress in cool-season crops and low temperatures combined with wetter circumstances may exacerbate fungal infections (Rana et al. 2016).

6.1.3 Importance in the Era of Changing Climate and Growing Population

To ensure food security for nine billion people, food production must be doubled by 2050. Efforts to increase global agricultural production have been hampered by recent climate changes resulting in rising sea levels, increased CO_2 concentrations, drought, floods, storms, extreme temperatures, glacier melting, reduced drinking water availability and so on (Bohra et al. 2019). Raise in temperature followed by rainy season have consistently exceeded the average temperature rise in India over the last four decades. This has harmed cereal and pulse yields, particularly those of pigeonpea, chickpea, wheat and rice. Pigeonpea cultivation in semiarid or arid regions; poor and marginalised environments allows it to thrive in harsh environmental conditions. Pigeonpea's nitrogen-fixing ability, like that of other grain legumes, allows for improved soil health and it will be especially helpful for non-leguminous crops during crop rotation (Yadav 2017).

6.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

Tough conventional breeding methods provide breeding opportunities against adverse stress conditions; crop duration and response, as well as genetic resources amenability, indicates the time taken to develop climate smart genotypes/variety is longer. Based on heritable and non-heritable trits and their interactions with different

environmental factors, selection was made in traditional breeding (Saxena et al. 2021a); contrast to this abiotic stresses screening pose challeges as it requires a variety of climatic factors, which often complicated by significant variation in it's occurrence, duration and intensity. The recent advent of various genomics technologies has paved the way for rapid improvement in crop breeding, resulting in reduction of uncertain and complicated environmental effects. In this regard, climate-smart pigeonpea may benefit greatly from advanced genomic technologies (Bohra et al. 2014, 2019, 2020). Currently, researchers are supported with advanced applicatons such as Genome Sequencing (GS), whole-genome re-sequencing (WGRS), speed breeding in pigeonpea with deployment of rapid generation advancement (RGA) technologies, marker assisted selection (MAS), marker assisted back cross (MABC) and early generation selection (EGS), etc., because high-density genotyping assays and available high-throughput phenotyping platforms helps in the identification of number of useful nucleotide sequence variations across the pigeonpea genome. Recently MOOB (multi-objective optimized genomic breeding) provided the oppertunity to regulate inbreeding rates and to improve the multi-trait sustainable selection process. MOOB in pigeonpea can be used in defining the training population for the GS and this could provide the rapid and sustainable genetic gains to develop climate resilient genotypes (Bohra et al. 2020). In this regard, the numerous abiotic stress management strategies for the development of climate resselient pigeonpea cultivars by involving wild relatives and landraces through plant breeding and recent genetic approaches are discussed in this chapter.

6.2 Descriptions of Different Abiotic Stresses

Pigeonpea has a wide range of environmental and cropping adaptations and is divided into four maturity groups; extra early (90–120 days), early (120–150 days), medium (150–200 days), and late (200–300 days). These differences in maturity have a direct impact on the crop's survival and fitness in various agro-ecological niches (Choudhary and Singh 2011). Each group is suited to a specific agro-ecosystem, as defined by altitude, temperature, latitude, and day length (Upadhyaya et al. 2013). Abiotic stress breeding is considered more difficult because of: (a) intricacy associated in abiotic stresses, (b) complex nature of abiotic stress tolerance in a genotype, (c) incidence of one stress more often in combination with the other, (d) differed intensity of abiotic stress under field condition (Choudhary and Vijayakumar 2012), (e) poor heritability of abiotic resistance. Hence, real time quantification of abiotic stress is essential in pigeonpea which is not well documented as compared to cereals. Drought resistance, WL, salinity tolerance, temperature tolerance (low/high), aluminium (Al) toxicity and photoperiod were considered as major stresses in pigeonpea, while metal toxicity and ultraviolet irradiation were identified as minor stresses.

6.2.1 Drought Resistance

Pigeonpea is the primary source of protein for a billion people in the semi-arid tropical (SAT) regions of the world (Sinha et al. 2013). Inherently pigeonpea is regarded as one of the potential pulse crops to sustain prolonged drought and heat stress. However, because of its wide maturity group and arid/semi-arid cultivation practices and occurrence of terminal drought stress can lead to potential yield reduction. The maintenance of water uptake under drought condition depends on several properties concerning plant roots such as root size, root density and size of xylem vessel and its efficiency etc., which in turn contributes more yields by increasing the water uptake from a deeper layer of soil (Choudhary et al. 2014).

Analysis of drought condition depends on osmotic adjustment (OA), relative water content (RWC) of leaves and dehydration tolerance of a genotype (Choudhary et al. 2011 and Sulthana et al. 2014). Lopez et al. (1996) in pigeonpea reported genotypic variations for drought tolerance. This variation was linked to the capacity of resistant genotypes to sustain efficient dry matter during droughts. Chauhan et al. (2002) identified ICPL 88,039 a promising short duration genotype that show a modest level of drought resistance. This genotype is suitable to grow in post rainy season after rice cultivation in Sri Lanka (Saxena 1999) and in Philippines (K.B. Saxena, unpublished data). Pigeonpea roots received slightly more total dry matter (TDM) than cowpea or soybean roots. Deshmukh and Mate (2013) reported that most physiological parameters appear to be ratios; genotypes selected based on their desirable estimates does not always result in the identification of genotypes with superior drought stress reproduction.

Based on research reports, conventional screening methods may not add solutions in a short duration. Pigeonpea genome sequencing has provided an excellent forum for studying the functional expression of any candidate gene(s) that can be used to mitigate abiotic stresses in a precise and faster manner. Using the three related algorithms; BestKeeper, geNorm, and NormFinder, a systematic study of a widely used total of 10 candidate housekeeping genes was selected and evaluated in 12 different sample for drought stress situation (early and late). The analysis of the datasets showed a series of stable housekeeping genes may be used as an internal control for gene expression studies in crop improvement (Sinha et al. 2015a, b, c). In a study by Niu et al. (2021) on ATP-binding cassette (ABC) transporter; a class of proteins that play a vital role in the physiological processes of growth and development in plants, a total of 51 ABCG transporters responding to abiotic stress were identified and divided into two subgroups: white-brown complexes (WBC) and pleiotropic drug resistance complexes (PDR). The analysis of protein structure and gene structure shows the presence of cis-elements in pigeonpea ABCG transporters and the highly conserved NBD domain determines the important function of the ABCG transporter. The initial results revealed that ABCG transporters are more effective in the abiotic stress resistance in pigeonpea.

6.2.2 *Waterlogging*

Waterlogging refers to soil saturation with water. Some crop plants including rice tolerate this stress by the virtue of their special character (Choudhary et al. 2015). However, warm (rainy) season pulses (e.g., pigeonpea, mungbean, urdbean, etc.) often experience WL especially at the seedling stage and the duration may vary from hours to a few days (Fig. 6.1). Under the waterlogged condition, oxygen diffusion rates (ODR) in flooded soil is about 100 times lower than air (Kennedy et al. 1992), reducing transpiration rates and net photosynthesis (Bohra et al. 2019). Takele and McDavid (1995) reported that chlorosis, senescence and abscission of lower leaves are a result of WL. Chlorosis of younger terminal leaves has been reported as the first visible symptom of WL (Hingane et al. 2015). Setter and Belford (1990) reported reduced growth, premature senescence and leaf drop as other visible symptoms of damage in waterlogged plants. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has identified 23 promising accessions for WL after a multi-disciplinary evaluation of core/minicore collection (Upadhyaya et al. 2016). Interestingly, resistance to WL is governed by a single dominant gene (Perera, et al. 2001; Sarode, et al. 2007) and its incorporation is comparatively easy. Sultana et al. (2013a, b) used a seed submergence treatment for 8 days (192 h) to measure germination rates (at 25.2 °C) in the dark under controlled conditions.

Kumar et al. (2020) tested 60 pigeonpea genotypes for submergence tolerance during the seed stage in the lab over three different time periods; 120, 144 and 168 h. Based on these conclusion, WL tolerant 40 genotypes were selected for seedling stage screening at field level by flooding the field at knee high stage (70 days after germination). WL tolerance using morpho-physiological, biochemical and molecular traits estimated for selected 20 genotypes. Six different genotypes were considered for random amplified polymorphic DNA (RAPD) primers amplification based on pot results. Mallikarjuna et al. (2017) and Hingane et al. (2015) reported that advanced breeding lines derived from a cross with *C. acutifolius* were screened under WL conditions; they showed the formation of lenticels above the water surface. This unique trait improves the survival rate of pigeonpea lines that are susceptible to WL.

In terms of pigeonpea metabolic responses in flooded conditions, WL-tolerant genotypes (ICPL 84,023 and ICP 301) showed higher enzymatic activity and gene

Fig. 6.1 Pigeonpea germplasm screening at the vegetative stage in a man-made pond (**a, b**) and in the field (**c**) at ICRISAT, Patancheru

expression for alcohol dehydrogenase (ADH) and sucrose synthase (SuSy) than susceptible genotypes (ICP 7035 and Pusa 207) (Kumutha et al. 2008). This suggests that pigeonpea WL tolerance is due to SuSy activity, which provides reducing sugars during glycolysis and ADH, which allows for the reduction of nicotinamide adenine dinucleotide (NADH), allowing for more efficient glycolysis and the maintenance of sufficient energy and sugar reserves under oxygen limiting conditions.

The impact of WL on susceptible (MAL 18) and resistant 117 (ICPL 84,023) genotypes of pigeonpea were studied by Bansal and Srivastava (2015), who found that both genotypes showed a decrease in CO_2 exchange rates, stomatal conductance, transpiration rates, and intracellular CO_2 concentrations. ICPL 84,023 was able to survive under waterlogged conditions due to its high carboxylation efficiency, increased chlorophyll content, starch availability, ADH activity and membrane stability. Duhan et al. (2017) reported that the combined effects of WL and salinity are more harmful to pigeonpea than any of the individual stresses alone.

6.2.3 Salinity Tolerance

Globally, over 100 Mha of arable land suffer from increased soil salinity, especially in irrigated areas (Saxena 2008). Salinity pressure negatively affects plant growth at all stages, with most of the sensitive periods being seedling and reproductive stages of the crop (Munns and James 2003). Suplus salts in soils can induce osmotic stress in plants and toxic level occumulation within cells interfere with mineral nutrient uptake (Chikelu et al. 2007). Plant height, leaf area, leaf area index (LAI), crop growth rate, net assimilation rate, TDM production and seed yield are all reduced in pigeonpea plants with elevated NaCl (15 m mhoes/cm^2) concentrations (Joshi and Nimbalkar 1983).

Srivastava et al. (2006) reported that tolerance to irrigation with 75 mM NaCl solution was tested on a pigeonpea mini-core collection, as well as in wild relatives and field collections are predominantly from saline areas, the results indicated that, the collection from Bangladesh, Indonesia and India's coastal areas had comparatively high levels of salinity resistance. In the shoots of the resistant pigeonpea genotypes, there was more biomass and less Na content. *C. sericeus, C. acutifolius, C. platycarpus,* and *C. scarabaeoides* were found to be resistant to salinity among the wild species. Widely accepted cultivars viz., C 11 (Chauhan et al. 2002), UPAS 120 (Promila and Kumar 1982) show resistance to salinity. Subbarao et al. (1991) investigated the salinity tolerance of different pigeonpea genotypes and wild relatives (*Atylosia*, *Rynchosia* and *Dunbaria*) and confirmed that the wild relatives were more tolerant than the cultivated genotypes. The hybrids derived from *C. platycarpus* and cultivated pigeonpea for biotic and salinity stress were studied by Mallikarjuna et al. (2011) and results were promising in advance generation progeny lines. In another experiment by Subbarao et al. (1990) the transfer of salinity tolerance from *C. albicans* to cultivated genotype was possible because tolerance in this wild species is articulated as a dominant genetic trait. During germination, Karajol and

Naik (2011) measured salinity tolerance in ten pigeonpea varieties at different mM NaCl concentrations. Salinity had little effect on germination percentage, but it did delay germination at higer concentration.

Plant biotechnology has been used to improve salinity tolerance. Genes encoding enzymes required for the biosynthesis of various osmoprotectants, as well as those encoding enzymes for modifying membrane lipids, late embryogenesis abundant (LEA) proteins and detoxification enzymes have been transferred. The potential of stress-inducible transcription factors has been demonstrated (Sharma and Lavanya 2002). Transgenic rice produced by Mellacheruvu et al. (2016) conferred resistance to the fungus *Magnaporthe grisea* and tolerance to drought, salinity and heat by expressing the *CcHyPRP* gene from pigeonpea.

To find candidate genes for various stresses, functional genomics approaches were used. Identified genes needs validatation and these candidate genes could be used to improve GAB in pigeonpea by providing multiple stress resistance (Pazhamala et al. 2015). Ectopic expression of *C. cajan* hybrid proline rich protein (*CcHyPRP*), *C. cajan* cyclophilin (*CcCYP*) and *C. cajan* chilling and drought regulatory (*CcCDR*) genes in Arabidopsis have been associated with distinct tolerance, increased plant biomass and photosynthetic rates under PEG/NaCl/cold/heat stress conditions (Sultana et al. 2014). The role of WRKY genes in various stress tolerance mechanisms is well-known. In response to salinity and drought stress treatments, the expression patterns of selected CcWRKY genes were investigated in two genotypes, Asha (ICPL 87,119) and *Rhynchosia minima* (wild).

6.2.4 Temperature Tolerance

Pigeonpea is widely grown between 30° N and 35° S latitude in Asia, East Africa and Central America in the tropics, subtropics, and warmer equatorial regions (Sardana et al. 2009). Pigeonpea can withstand high temperatures and water scarcity; it is a popular crop in arid and SAT regions of the world (Mir et al. 2017). The vegetative phase is usually associated with relatively long days at higher latitudes (Reddy and Virmani 1981) and warm temperatures at lower latitudes (Omanga et al. 1995). Due to low temperature, moisture stress, internal injury, the production of reactive oxygen species (ROS), photosynthesis is completely inhibited. Cold tolerance in pigeonpea screened using lines that survive at temperatures as low as 0 °C and have normal morphology (Rana et al. 2016; Sandhu et al. 2007). However, before using these lines in crop improvement, it must be determined whether the survival advantage in these genotypes helps with reproduction (Sulthan et al. 2014). The number of blossomed flowers, the initiation and development of floral buds and pod setting at low temperatures can all be used as morphological markers to distinguish between sensitive and tolerant pigeonpea genotypes (Choudhary et al. 2007; Singh and Singh 2010).

Cold temperatures tolerance is influenced by a many number of factors, including the range of temperature drops, the duration of low temperatures, genotype, plant

age, soil moisture and so on. Temperature is the most important of these factors in pod development (Turnbull et al. 1981; Nayyar et al. 2005). Its difficult to asses the damage caused by frost due to its patchy nature and to predict the compensation which may occur during grain filling (Yong et al. 2002). In this cotext MAS can be efficiently engaged to develop frost tolerant genotypes (Singh et al. 2012a, b).

Durgesh et al. (2019) screened 302 germplasm/lines of pigeonpea durng 2016–18, including varieties and advanced materials, for frost injury and they were able to identify tolerant and susceptible lines. To study the nature of frost injury and mode of inheritance, a highly frost tolerant (T) (insensitive) line ICP 10,509 was crossed to a susceptible (S) (sensitive) line ICP-11182 based on screening data, thhe F_1 hybrid was frost tolerant, with only 1–2 leaves showing symptoms (score 0–1), indicating that the trait was dominant. The expected ratio of 3(T): 1(S) (P value = 0.06027) fits well in the F_2 population segregating into tolerant (216 plants) and susceptible (91 plants), indicating that frost tolerance (insensitivity) is controlled by a single dominant gene.

Heat stress is defined as a temperature rise that exceeds a threshold level for a long enough period of time to cause irreversible damage to plant growth and development. Heat stress causes morphological, physiologiacl, metabolic and reproductive changes in plant (Kaushal et al. 2016). While, the heat shock is defined as a transient increase of 10–15 °C above ambient temperature that may cause minor damages during crop establishment and these changes are improve when the temperature return to normalcy. Heat stress, on the other hand, is a complicated function of temperature intensity (in degrees), duration and rate of increase in temperature (Wahid et al. 2007). It is critical to find genotypes that can set seeds at higher-than-optimal temperatures. In doing so, caution should be exercised with the experimental approach, as simply delaying the planting date to ensure that reproductive development occurs at high temperatures including the amount of radiation received by the crop, which help in the selection of heat tolerant genotype (Gull et al. 2020). Basu et al. (2016) reported that at higher temperatures (≥38 °C), pigeonpea pollen sterility was found to be very high. Few accessions of *C. scarabaeoides* that flowered and set pods in the temperature range of 20–40 °C could be used as donors for introgressing heat tolerance in pigeonpea cultivars.

Danekar et al. (2014) identify and characterize the Hsp100 family genes [Chaperone protein (ClpB)] which are major and critical genes required for acquired thermo tolerance. Five Hsp100-gene sequences were discovered after genome-wide characterization through in silico method. The expression of these genes was observed in control, 2, 6, and 24 h after heat stress, signifying that they are heat inducible nature. Two Clp genes, which are targeted to the chloroplast, show constant expression under heat stress and could be important components of the heat stress reaction in pigeonpea. Candidate gene based approch was tried to find additional genes information, out of 23 genes of *Glycine max* Hsp100, 20 were expressed in pigeonpea showed the presence of more Hsp100 family genes in the genome that need further investigation. This research will aid in the understanding of the molecular components that govern pigeonpea thermo tolerance. Niu et al. (2021) discovered CcABCG28, low-temperature stress-related cis-acting elements and these elements were highly

conserved with short CCGAAA sequences. They found that the expression of CcABCG7 was downregulated under high-temperature stress, but that the expression of CcABCG7 in leaves was upregulated.

6.2.5 Photoperiod

Pigeonpea photoperiod sensitivity has been a key factor in determining its growth and development and it requires shorter days and long hours of darkness for flower induction (Silim et al. 2006; Vales et al 2012). Traditional pigeonpea cultivars and landraces are photoperiod sensitive, limiting their adaptation to temperatures between 30° N and 30° S. Germplasm has a wide range of maturity duration between 90–300 days and this variation is almost continuous in nature (Saxena 2008). Since, the phenology of pigeonpea plants are heavily influenced by photoperiod, temperature and their interactions, the classification based on maturity groups may not hold true in every location and it is crucial in the process of evolution of the various pigeonpea crop production systems which have been established so far. Hence, breeders need to know the flowering behavior of parental lines of respective location before selecting them for hybridization and implementation of appropriate selection schemes (Byth et al. 1981).

The photoperiod sensitive reaction is linked not only to days to flowering but also the amount of biomass produced (Wallis et al 1981). Plant physiological dwarfing occurs when photoperiod sensitive types are sown during the shortest day of the year, necessitating increased plant populations to maximize yields as reported by Spence and Williams (1972). Turnbull et al. (1981) investigated the effect of temperature and photoperiod on pigeonpea floral development. Under the 24/16 °C temperature regime, they discovered a few day-neutral cultivars. The time it took from floral initiation to flower opening (rate of floral primordia development) ranged from 40 days at 24/16 °C under an 8 h photoperiod to 22 days at 32/34 °C under a 16-h photoperiod. Dvelopment of suitable genotype for rainfed cropping is on priority hence, breeders have developed gentypes matures in between 90–120 days and insensitive to photoperiod.

Photo insensitive cultivars have shown adaptation to latitudes ranging from the equator (Kenya) to 46° N (Prosser, USA) and 45° S in New Zealand. Pigeonpea cultivars ICPLs 83,105, 85,010, and 85,030 produced 1,500–2,500 kg ha^{-1} grain yield at Prosser (Saxena 2000). The suitability of such cultivars offers opportunities for extending pigeonpea cultivation into new niches.

Under a 16 h photoperiod induced condition Saxena et al. (1983a, b), were able to identify three major genes, PS1, PS2 and PS3 which control flowering in the photoperiod sensitive parent MS4A. PS3 overrides PS2's expression, and PS2 overrides PS1 in the hierarchy and findings indicate that more research is needed to fully comprehend the effects of photoperiod and temperature on flowering in genotypes of various maturity groups. Extra-short pigeonpea genotypes developed by ICRISAT, escape

drought and are relatively less sensitive to photoperiod than traditional varieties with longer growth cycles.

The molecular mechanism involved in flower initiation is well characterized in Arabidopsis [long day plant (LD)] and rice [short day plant (SD)] (Jeong and Clark 2005; Izawa 2007). Flowering largely depends upon the expression of *Flowering Locus T* (*FT*) in Arabidopsis and *Hd3a* and *RFT1* in rice which encode florigen, a systemic signaling molecule required for flower induction. It is transcribed and translated in the phloem companion cells of leaves to the shoot apex (Corbesier et al. 2007; Tamaki et al. 2007; Cheng et al. 2018). It forms a 'florigen activation complex' (FAC) at shoot apex after interacting with 14–3–3, a class of TPR proteins and FD proteins, which induces transcription of floral meristem identity genes similar to *APETALA1* (*AP1*)-like and *LFY* gene, leading to flower development (Taoka et al. 2011). To understand the structural and regulatory nature of the *FT* gene in pigeonpea (Tribhuvan et al. 2020), a genome-wide survey was carried out, revealing the presence of 13 PEBP (phosphatidyl ethanolamine-binding protein) (*FT*) family genes in *C. cajan*; gene expression profiling of these 13 PEBP genes in the different 30 tissues of *C. cajan*, *CcFT6* and *CcFT8* were found to be probable *FT* genes responsible for the production of florigen since both showed expression in reproductive leaf tissue.

6.2.6 Al Toxicity

After oxygen and silicon, Al is the third most abundant element in the earth's crust. The presence of toxic Al^{3+} in acidic soils (pH 5.0) is a production limitation over the globe (Kochian et al. 2005; Vitorello et al. 2005). Al toxicity is a problem in 30% of arable soils, affecting crop yields (Campbell et al. 1988). The inhibition of root elongation is the first indication of Al toxicity, which has been attributed to different mechanisms, including Al interactions with the plasma membrane (Pineros and Kochian 2001) or the symplast (Kochian 1995). Root growth is severely hampered by Al toxicity, which also interferes with water and mineral nutrient uptake. Excess Al disrupts cellular redox balance and increases the production of ROS, leading to oxidative damage (He et al. 2014). Tolerant genotypes retained normal physiological function at toxic Al levels (Choudhary and Singh 2011). The Al toxicity screenig in grain legumes are limited to seedling screening and few in number (Choudhary et al. 2011).

The majority of grain legumes are sensitive to Al toxicity. There is a lot of deviation in plant species and genotypes within species when it comes to Al toxicity tolerance (Kinraide et al. 1985; Singh and Choudhary 2009). Because of the large temporal and spatial variation in acidic soils, a reliable ranking of tolerance in the field is difficult (Choudhary et al. 2011). Furthermore, when numbers of genotypes are more to be evaluated, screening at field level may not be cost effective (Garcia et al. 1979). Additionally, the results by the solution culture screening method on par with results obtained by means of field screening (Urrea-Gomez et al. 1996), indicating reliability

of laboratory screening method. The lab experiment results through hydroponic and sand assays, hematoxylin staining, and root re-growth were nearly identical to screen for Al tolerance in pigeonpea. Singh and Choudhary (2009) evaluated 32 pigeonpea genotypes for Al toxicity tolerance. In the shoots of different pigeonpea genotypes, no obvious signs of Al toxicity were found. Root growth was, however, restricted. By inducing the activity of antioxidant enzymes, 24-epibrassinolide removes this toxicity and gives normal yield (Sri et al. 2016a, b). Al-induced excretion from roots is one of the mechanisms of Al tolerance in pigeonpea that has been revealed by biochemical analysis (Daspute et al. 2017).

6.2.7 Traditional Breeding

Improvement of pigeonpea was limited due to involvement of local genotypes, as well as their identification for high yield ability and biotic resistance breeding (Wanjari et al. 2016). Pigeonpea breeding has been more difficult than other food legumes due to a variety of crop-specific traits (Varshney et al. 2010a, b, c, d). Pigeonpea is an often cross-pollinated crop, with natural outcrossing rates ranging from 20 to 70%, limiting the use of efficient selection and mating designs like in self-pollinating species. Despite having a 30% yield advantage over non-hybrids, GMS-based hybrids were unable to be commercialized due to the high cost of hybrid seed production. Higeher yield realization in the GMS hybrids compared to conventional varities, it promted to the development of more efficient cytoplasmic-genetic male-sterility (CGMS) system (Tikka et al. 1997; Saxena and Kumar 2003; Wanjari and Patel 2003). The first CMS-based hybrid GTH-1 was released in India in 2004 as a result of an intensive hybrid development program at ICRISAT in collaboration with its partners. Another cytoplasmic male-sterility (CMS) based pigeonpea hybrid, ICPH 2671, was developed at ICRISAT in 2005 (Saxena 2008) using *C. cajanifolius* (A4 cytoplasm) and has been released as "Pushkal" by Pravardhan Seeds for cultivation in different parts of India. Pigeonpea yield plateauing has been a cause of concern for breeders for the past 6–7 decades. During this time, serious breeding efforts resulted in a variety of high-yielding, disease-resistant cultivars. At ICRISAT, a comprehensive multidisciplinary evaluation of the core/minicore collection resulted in the identification of promising accessions for salinity (16), WL (23), high yield (54), high zinc (15) and iron content (15), whereas NARS identified trait-specific germplasm for early maturity (8), high seed yield (2) (Upadhyaya et al. 2016). Despite the high genetic diversity of wild relatives, their use has been limited due to a lack of appropriate information on the presence of useful traits (Goodman 1990). A close association between desired and undesirable features frequently impedes the transfer of target genes from wild to cultivated types. Aside from them, the time it takes to transfer a useful feature and select for it in combination with a high yield is time consuming.

6.2.8 *Limitations and Prospect of Genomic Designing*

Despite the abundance of germplasm, its application in pigeonpea improvement is limited and unexplored (Majumder and Singh 2005; Pazhamala et al. 2015). Pigonpea was the first orphan and non-industrial legume crop to have a draft genome sequence, which was published in 2012 (Venkata et al. 2019). Because it uses untapped genomic resources for trait mapping and molecular breeding, pigeonpea is a resource-rich legume crop. SSR markers (Saxena et al. 2010a, b; Bohra et al. 2011a, b; Dutta et al. 2011; Mir et al. 2017), Diversity array technology (DArT) markers (Yang et al. 2006, 2011), single feature polymorphism (SFP) and single nucleotide polymorphism (SNP) genotyping platforms (Varshney et al. 2012a, b; Saxena et al 2014) have been used to develop high density genetic maps in pigeonpea. These resources help in increasing the chances of discovering genes/QTLs that are responsible for important targeted traits, resulting in crop genetic improvement. In addition, markers and candidate genes responsible for traits such as flowering time, fertility restoration, wilt and SMD resistance (Mir et al. 2017), yield and phenology in pigeonpea have been recognized by association mapping, marker-based QTL mapping, candidate gene-based QTL mapping, transcriptomics, and whole genome sequencing approch (Mir et al. 2017). Modern genomic tools viz, NGS, genome wide genetic markers and transcriptome/genome assemblies made it possible to create a diverse set of genomic resources (Venkata et al. 2019). Complementary support for NGS via genomic interference will add to breed the genotypes against abiotic stress at a faster rate and in a precise manner.

6.3 Genetic Resources of Resistance/Tolerance Genes

Pigeonpea belong to the Cajaninae subtribe of the tribe Phaseoleae, which is part of the Papilionoideae subfamily of the fabaceae family. *Cajanus* was once thought to be closely related to *Atylosia* and the subtribe Cajaninae had 13 genera. *Atylosia* was later merged with *Cajanus* based on morphological, taxonomic, and cytological evidences. In the subtribe Cajaninae (van der Maesen 1990), 32 species and 11 genera recognized in the genus *Cajanus*, mostly from India and Australia. *Cajanus cajan* is the only domesticated species in the Cajaninae subtribe. The genome of the pigeonpea is diploid ($2n = 2x = 22$), with a physical size of 0.853 pg (858 Mbp) (Greilhuber and Obermayer 1998). With the exception of *C. kertsingii*, the only African species with a haploid chromosome number of 16 (Gill and Hussaini 1986), all wild pigeonpea relatives have the similar chromosome number as the cultivated type. Except the presence of a prominent strophiole, *C. cajanifolius* (formerly *Atylosia cajanifolius*) resembles the cultivated pigeonpea in all morphological attributes. Apart from that, these two species easily cross and produce viable F_1s, implying a high level of genomic homology between them. *C. cajanifolius* was thus considered to be the most likely progenitor of cultivated pigeonpea based on several morphological, biochemical,

Table 6.2 *Cajanus* species gene pool details (Wanjari et al. 2016)

Primary gene pool	Secondary gene pool	Tertiary gene pool	Quaternary gene pool
C. cajan and its related land races	*C. acutifolius* *C. albicans* *C. cajanifolius* *C. lanceolatus* *C. laticepalus* *C. lineatus* *C. reticulatus* *C. scarabaeoides* *C. sericeus* *C. trinervius*	*C. aromaticus, C. crassicaulis,* *C. cinereus C. marmoratus,* *C. gandiflorusC. crassicaulis,* *C. goensis, C. heynei, C. kerstingi,* *C. lanuginosus, C. pubescens,* *C. mareebensis, C. lanuginosus* and *C. pubescens* *C. mollis, C. rugosus, C. volubilis,* *C. platycarpus, C. niveus,* *C. rugosus, C. elongates,* *C. villosus, C. confertiflorus, C. visidus,*	*Adenodolichos* *Baukea* *Bolusafra* *Carissoa* *Chrysoscias* *Dunbaria* *Erisema* *Flemingia* *Paracalyx* *Rhynchosia*

and cytological investigations (Pundir and Singh 1985). Harlan and de Wet (1971) established the concept of different gene pools to provide a practical guide based on genetic perspective of cultivated and wild relatives, based on this primary, secondary and tertiary gene pools are the three major gene pools. According to the gene pool concept breeders should start with primary gene pool and work their way outwards, on the other hand, recent advancements in crop improvement enable the exercise the benefits from the secondary, tertiary and quaternary gene pools in pigeon pea (Table 6.2) (Fig. 6.2).

6.3.1 Primary Gene Pool (GP1)

The GP1 of pigeonpea consists of all the cultivars or landraces of *C. cajan*. Several gene banks around the world accomodate high number of germplasm collections. ICRISAT, with the largest germplasm collection (Table 6.3), has 13,632 active accessions, including 8,215 landraces, 4,795 breeding material and 67 cultivars and advanced lines (Upadhyaya et al. 2007; Saxena 2008) in its gene bank. Despite the availability of large collections, the use of germplasm in breeding programs has been limited. To speedup the utilization of germplasm in breeding programs, core and mini core concepts have been proposed. At ICRISAT, the core and mini core collections consists of 1,290 and 146 accessions, respectively.

Despite the fact that wild *Cajanus* species have a lot of diversity, they aren't used enough in pigeonpea breeding programs. The main stumbling block is linkage drag, which is characterized by extreme incongruity barrier stuck between cultivated and

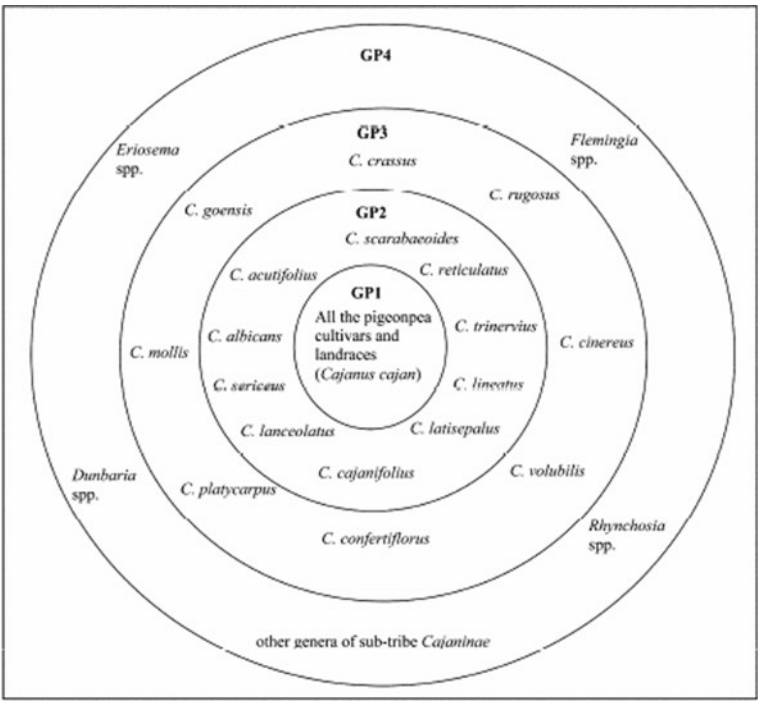

Fig. 6.2 Based on crossability relationships, the entire pigeonpea germplasm is divided into four gene pools (GPs). The primary gene pool includes cultivated types (GP1). Remaining Cajanus species group are found in the secondary (GP2) and tertiary (GP3) gene pools. Other related genera form the quaternary gene pool (GP4), which defines the genetic resource boundary (Bohra et al. 2010)

wild species. Pre-breeding offers a unique opportunity to expand the primary gene pool by utilizing genetic variability found in wild species and cultivated germplasm in such situations.

6.3.2 Secondary Gene Pool (GP2)

Cajanus sericeus, C. albicans, C. lineatus, C. trinervius, C. cajanifoliusv and *C. scarabaeoides* are the species from GP2 that have shown crossability with the cultivated type (Reddy et al. 1981). When the cultivated type was used as a female parent, interspecific hybridization was successful and reciprocal crosses with *C. cajanifolius, C. lineatus* (Pundir and Singh 1985) and *C. scarabaeoides* were also successful (Ariyanayagam and Spence 1978). *Cajanus cajanifolius, C. scarabaeoides* and *C. sericeus* were used as donor parents in interspecific hybridization and hybrids from *C. cajan* × *C. sericeus* and *C. cajan* × *C. cajanifolius* were successfully produced.

Table 6.3 Pigeon pea germplasm is available at the ICRISAT gene bank

Sl. No	Material	Number of accessions	References
a	Active collection	13,632	Upadhyaya et al. (2008)
	1. Landraces	8,215	Upadhyaya et al. (2011a, b)
	2. Breeding material	4,795	Upadhyaya et al. (2011a, b)
	3. Wild relatives	555	Upadhyaya et al. (2007)
	4. Advance cultivars	67	Upadhyaya et al. (2010)
b	Core collection	1,290	Reddy et al. (2005)
	1. Breeding material	466	Upadhyaya et al. (2007)
	2. Landraces	810	Upadhyaya et al. (2007)
	3. Advance cultivars	9	Upadhyaya et al. (2007)
	4. Others	5	Upadhyaya et al. (2007)
c	Mini core collection	146	Upadhyaya et al. (2006)
d	Base collection	11,794	Upadhyaya et al. (2008)

a—Collections for the bredeers distribution
b—10% of active collection is represented
c—1% of active collection is represented
d—for long term storage purpose

In pigeonpea, interspecific hybridization has resulted in the recovery of some novel plant types. The cross between *C. cajan* and *C. acutifolius* produced hybrids with improved pod borer resistance (Mallikarjuna and Saxena 2002). Additionally, distant hybridization between *C. cajan* and *C. scarabaeoides* resulted in isolation of ICPL 87,162, a line with a protein content increase of more than 27% (Reddy et al. 1997).

6.3.3 Tertiary Gene Pool (GP3)

There are 555 wild relatives in the ICRISAT gene bank with 57 wild species. Inbreeding has seen a random loss of alleles during the process of evolution, domestication and adoption, lead to genetic erosion (Tanksley and McCouch 1997). As

a result, the cultivated gene pool has a low level of genetic diversity (Miller and Tanksley 1990). Hence, crop wild relatives must be part of breeding programme to broaden the cultivated gene pool's narrow genetic base (Table 6.4).

Some crop wild relatives of pigeonpea, such as *Atylosia* and *Rhynchosia* were also crossed with *C. cajan* and the resulting hybrids, F_2 and F_3 families were evaluated for physiological efficiency and agronomic superiority (Pundir and Singh

Table 6.4 Genes for resistance/tolerance to various abiotic stress traits present in wild species

Important abiotic stress traits	Wild species	References
Cold tolerance	*C. confertiflorus*	Sharma and Upadhyaya (2016a, b)
	C. mollis	
Drought tolerance	*C. albicans*	Mallikarjuna (2003)
	C. acutifolius	Srivastava et al. (2006)
	C. lineatus	Mallikarjuna (2003)
	C. scarabaeoides	Mallikarjuna (2003)
	C. sericeus	Mallikarjuna (2003)
	R. rothii	Pundir and Singh (1987)
	C. cinereus	Sharma and Upadhyaya (2016a, b)
	C. lanceolatus	
	C. latisepalus	
Early flowering	*C. platycarpus*	Mallikarjuna et al. (2006)
	R. rothii	Pundir and Singh (1987)
Heat tolerance	*C. acutifolius*	Srivastava et al. (2006)
	C. cajanifolius	Sharma and Upadhyaya (2016a, b)
	C. cinereus	
	C. lanceolatus	
	C. latisepalus	
High precipitation,	*C. crassus*	
	C. lineatus	
Photoperiod insensitivity	*C. platycarpus*	Mallikarjuna et al. (2006)
	R. rothii	Pundir and Singh (1987)
Salinity tolerance	*C. acutifolius*	Srivastava et al. (2006)
	C. scarabaeoides	
	C. sericeus	
	C. platycarpus	
Super-early flowering (between 34–40 days)	*C. platycarpus*	Sharma and Upadhyaya (2016a, b)
Temperature variation/seasonality	*C. crassus*	
Waterlogging	*C. crassus*	
Waterlogging, drought	*C. lineatus*	

1986). *C. platycarpus*, a wild species from GP3, has been actively engaged in trait introgression because it possesses many important traits such as photoperiod insensitivity, extra-early flowering and maturity, productive flowering and pod setting, annuality, rapid seedling growth, salinity tolerance and resistance to phytophthora blight, cyst nematode and Helicoverpa (Mallikarjuna et al. 2006). Interspecific hybridization with *C. platycarpus* as donor parents resulted in hybrids with shrivelled and non-viable seeds, indicating the presence of some crossability barriers (Yadav and Padmaja 2002). Hybrids between *C. platycarpus* and cultivated pigeonpea were created by using embryo rescue techniques to prevent embryo abortion, followed by chromosome doubling with colchicine treatment. Insect and disease resistance, as well as salinity tolerance, were demonstrated in advance generation progeny lines (Mallikarjuna et al. 2011).

6.4 Glimpses on Classical Genetics and Traditional Breeding

6.4.1 Classical Mapping Efforts

Different methods, such as morphological and/or phenotypic, biochemical, and molecular markers, can be used to explore genetic variability in crop species (Mehmood et al. 2008). Because it is simple and inexpensive, morphological characterization is considered a traditional method. It does not require any special equipment or procedures (Mehmood et al. 2008). Morphological characterization provides a picture of a crop species based on its phenotype, but it is heavily influenced by the environment (Abdi et al. 2002). In the past, morphological traits, both qualitative and quantitative used in pigeonpea improvement (Kallihal et al. 2016; Navneet et al. 2017). Breeders could use information on genetic diversity for distantly related genotypes to help them choose desirable parents for introgression (Kimaroa et al. 2021).

It was suggested that a more comprehensive classification depending on morphological and agronomic characters developed in order to effectively and economically maintain and use the world pigeonpea germplasm collections (>12,000 accessions) (Reddy 1990). The 12,153 pigeonpea accessions from 56 countries were divided into 14 groups, mostly based on their geographical origin. Based on different statistical parameters, Kimaroa et al. (2021) found significant variability in agro-morphological traits among 48 pigeonpea genotypes. Stem color, growth habit, flowering pattern, seed eye color, base flower color, pod from, base seed color and seed color pattern were the most important qualitative traits responsible for observed variability.

Isozyme analysis and comparative electrophoresis of general proteins have both been successful in determining genetic homologies and understanding phylogenetic relationships among related taxa of pigeonpea (Krishna and Reddy 1981). Electrophoretic studies of the seed proteins of cultivated species and four *Atylosia* species

by Ladizinsky and Hamel (1980) revealed a close affinity among these species, implying a polyphyletic origin of *C. cajun* from several *Atylosia* species. Singh et al. (1981), the major protein subunits of the cultivated and eight wild relatives including six *Atylosia* species were very similar. Krishna and Reddy (1981) used polyacrylamide gel electrophoresis and isoelectrofocusing to examine the esterase isozymes in seed extracts of *Cajanus cajan* and six *Atylosia* species, concluding that isozyme patterns were stable and accession specific.

The large amount of variation in the genus *Cajanus* as a whole and in cultivated species in particular says the cultivated species have a less genetic diversity. Molecular studies, on the other hand, have revealed that the cultivated species has extremely low levels of polymorphism in comparison to wild species (Yang et al. 2006). These findings suggest use of an interspecific cross and the development of a large number of markers (Odeny et al. 2009) while developing pigeonpea linkage map. The DArT markers involved to create the first generation pigeonpea linkage map or reference map for an interspecific mapping population (ICP 28ICPW 94) of 79 F_2 individuals by Yang et al. (2011).

Only a few hundred SSR markers were available for pigeonpea (Odeny et al. 2009; Saxena et al. 2010a, b). This situation made more difficult by low levels of genetic diversity within cultivated germplasm, necessitating large-scale development of SSR markers. SSR markers have traditionally been identified and developed using three approaches: (I) building an SSR-enriched library and sequencing SSR positive clones (Gupta and Varshney 2000), (ii) mining the EST (expressed sequence tag) transcript sequence generated by Sanger sequencing (Varshney et al. 2005) or short transcript sequences generated by NGS technologies (Varshney et al. 2009), (iii) mining the BAC (bacterial artificial chromosome)-end sequences (BESs) (Mun et al. 2006). Despite the labor-intensive and time-consuming nature of SSR enrichment and very low polymorphism levels of SSRs identified from transcript sequence mining, the first two approaches used to widen SSR markers in pigeonpea with some success. The SSR markers development from BESs overcomes the drawbacks of the first two approaches because a large number of SSRs will be identified quickly and genomic SSRs have a higher level of polymorphism than transcript-associated SSRs and also helps in development of combined genetic and physical maps (Mun et al. 2006; Shultz et al. 2007a; Schlueter et al. 2007).

For the construction of a reference genetic map, Bohra et al. (2011a, b) studied an inter-specific F_2 population derived from ICP 28 (*C. cajan*) and ICPW 94 (*C. scaraboides*) (Fig. 6.3). This pairwise comparison had the most polymorphic SSRs, which is consistent with a large genetic crosses group. Genotyping based on polymorphic data in the mapping population and marker segregation data was analysed using the goodness of fit test for a 1:2:1 segregation ratio. Starting with the 138 normally segregating markers at logarithm of odds (LOD) 5 and a minimum recombination fraction of 37.5, the genetic linkage map was built step by step. A total of 11 linkage groups were identified, and these are thought to correspond to *C. cajan* haploid chromosome set ($n = 11$). Bohra et al. (2012) were the first to report on the construction of a consensus genetic map in pigeonpea and the identification of QTLs using four intra-specific F_2 populations (ICPB 2049 $\times$ ICPL 99,050, ICPA

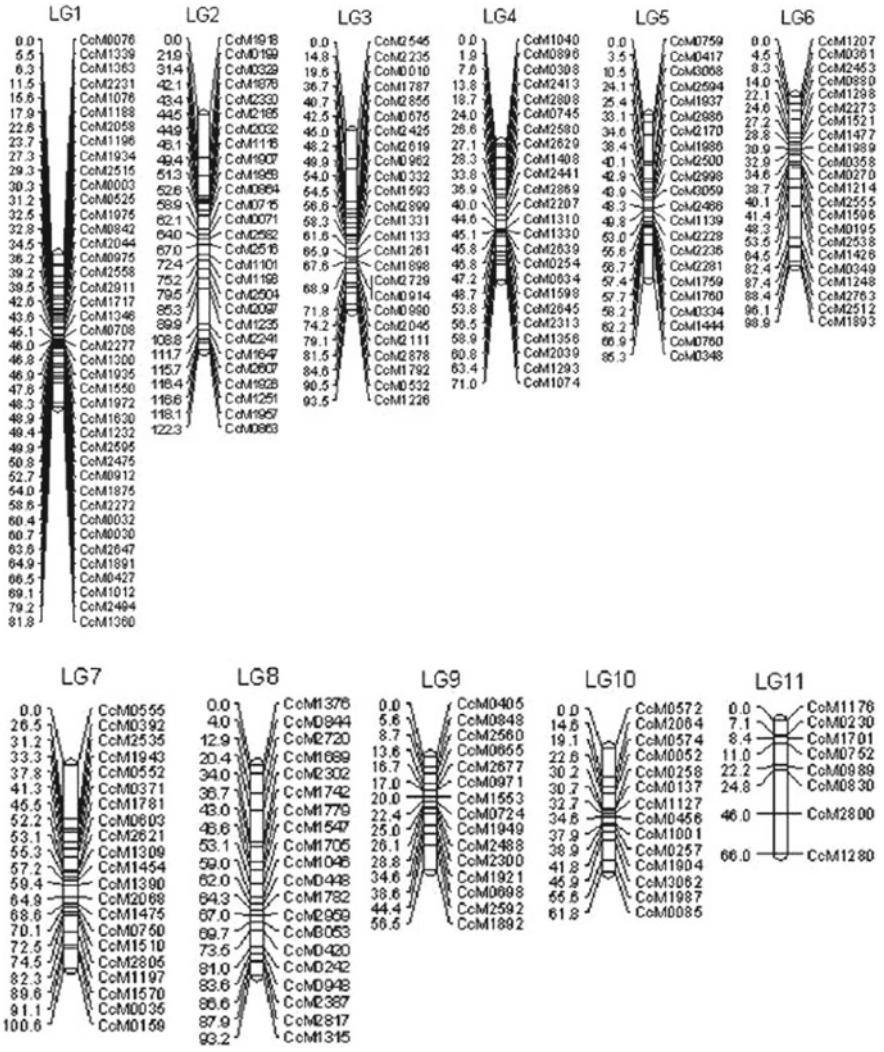

Fig. 6.3 A pigeonpea reference genetic map derived from an inter-specific F2 population (ICP 28 ICPW 94) (Bohra et al. 2011a, b)

2039 × ICPR 2447, ICPA 2043 × ICPR 3467 and ICPA 2043 × ICPR 2671; each with 188 individuals) for fertility restoration, three of which was created with 59–140 SSR loci. The pigeonpea genome was mapped using an SNP-based, high-density intraspecific consensus linkage map, which included 932 loci with 1.51 cM average marker interval. Three different intra specific F_2 mapping populations (Asha/UPAS 120, Pusa Dwarf/H2001-4, and Pusa Dwarf/HDM04-1) were used to construct the

component linkage maps using Illumina' Golden Gate assay and reduced representation RAD (restriction-site associated DNA) genotyping-by-sequencing (GBS) approaches (Arora et al. 2017).

6.4.2 *Limitations of Classical Endeavors and Utility of Molecular Mapping*

Pigeonpea yield has remained consistently low for decades due good number of reasons. Important agronomic traits in pigeonpea have yet to be figured out genetically and their molecular basis is unknown. Furthermore, pigeonpea from GP1 has a low level of DNA polymorphism and there are no validated molecular markers in this species (Odeny et al. 2009). The development of several interspecific and intraspecific maps has resulted from recent efforts to build a genetic map of pigeonpea. Using 554 DArT markers, the first interspecific map of pigeonpea was created, covering a total map distance of 451.6 cM (Yang et al. 2011).

Previous pigeonpea linkage maps mostly employed DArT, RAPD, ISSR and SSR markers. Although these markers are useful, some have dominant type inheritance (Arora et al. 2017); co-dominantly inherited markers are only precised once. The Indian Council of Agricultural Research (ICAR) launched the Pigeonpea Genomics Initiative (PGI) to address these issues as part of the Indo-US Agricultural Knowledge Initiative (AKI). By developing numerous SNP and SSR markers and moderately dense linkage maps to facilitate marker-assisted breeding (MAB) in pigeonpea. The draft genomes of the pigeonpea variety "Asha" were assembled using FLX 454 (Roche Inc., Germany) and Illumina (San Diego, USA) sequencing technologies (Singh et al. 2012a, b; Varshney et al. 2012a, b); however, due to the low polymorphism, it was not possible to map a large number of loci in a single mapping population. As a result, multiple mapping populations with an acceptable number of common markers are used to increase the number of mapped markers and construct a dense consensus map.

6.4.3 *Breeding Objectives*

The main breeding goal in pegionpea is to develop varieties resistant to an array of biotic and abiotic stresses. In addition to increasing tolerance to biotic stress (Saxena et al. 2016a, b), current breeding objectives for pigeonpea include increasing tolerance to abiotic stresses. Because of crop physiology is the least researched area in pigeonpea and limited information on physiology of various stresses leads to the poor performence of crop at field level (Saxena et al. 2016a, b). The severity, timing, and duration of the drought, on the other hand, determine the extent of yield losses (Chapman and Muchow 1985).

The photoperiod-insensitive and short-duration genotypes is on high priority for higher latitude in order to avoid dry matter partitioning, which affects unequal competition among developing pods and vegetative plant parts (Chauhan et al. 2002). According to Saxena et al. (2016a, b), three pigeonpea breeding milestones have been achieved. These include the creation of (i) medium maturing; disease resistant with enhanced yield and stability, (ii) high yielding early maturing pigeonpea cultivars for new production niches and (iii) a trend-setting hybrid breeding technology for breaking the decades-old low yield plateau.

6.4.4 Classical Breeding Achievements

The Coordinated Research Project made available to Indian farmers over 120 varieties of pigeonpea, ranging in maturity from early (120–140 days), medium (150–170 days), midlate (170–200 days) and late (more than 200 days) (Singh 2014). Farmers' established varieties and landraces were selected from local germplasm and promising genotypes were identified through multi-location testing (Wanjari et al. 2016). After analyzing a pigeonpea mini-core collection, 23 accessions (ICP 1279, ICP 4575, ICP 5142, ICP 6370, ICP 6992, ICP 7057 and etc.) were recognized as tolerant to WL conditions (Krishnamurthy et al. 2011) and 16 accessions (ICP 2746, ICP 3046, ICP 6815, ICP 7260, ICP 7426 and others for salinity tolerance (Srivastava et al. 2006). Identification of eight accessions (ICP 1156, ICP 9336, ICP 14,471, ICP 14,832, ICP 14,900, ICP 14,903, ICP 15,068 and ICP 16,309) for early flowering (85 days); three accessions (ICP 13,139, ICP 13,359 and ICP 14,976) for large seed size (>15 g/100 seed); and one accession (ICP 14,832) for early flowering (85 days) (ICP 8860) (Upadhyaya et al. 2010) were identified.

6.4.5 Limitations of Traditional Breeding and Rationale for Molecular Breeding

Saxena (2008) indicated that over the last few decades, significantly contributed for breeding to develop crop duration reduction, seed quality improvement and overcoming the constraints of major diseases like wilt and sterility mosaic. Even though yield per unit of land area has not significantly increased, these milestones have helped to increase pigeonpea production and area. Due to limited land resource and growing demand, researchers must now concentrate their efforts on improving yield genetically. Two possible methods for achieving a breakthrough in yielding ability are utilizing heterosis for yield and restructuring plant type for increased harvest index. But recognizing the presence or absence of a particular gene at the molecular level is independent of plant part; Gepts (1999) discussed the use of molecular markers to improve the efficiency of plant breeding programs. Molecular markers,

unlike morphological traits, are not affected by pleiotropic and epistatic interactions. Establishing linkage between a gene and its marker locus is thus the first step in molecular breeding. Specific DNA diagnostic tests can then be used to aid plant breeders in their selection. MAS is the process of identifying useful breeding lines with the help of linked molecular markers. Molecular markers are particularly useful for traits having low heritability where phenotypic selection would be poorly effective. Including landraces and wild progenitors in breeding programs, identifying QTLs and candidate genes associated with important agronomic could provide an excellent solution to ensure food security in the face of global climate change. New transcriptomic technologies have recently been developed to speed up the development of molecular markers and trait improvement programs in a variety of crop plants, it also offer a promising way to find novel functional and regulatory genes involved in stress adaptation that have been lost as a result of legume domestication. Likewise, more research into the role of miRNAs in posttranscriptional regulation, could aid in the genetic improvement of abiotic stress resistance in legumes (Abdelrahman et al. 2018). Recent advances in NGS-based genomic sequencing and the latest molecular breeding methodologies like genomic selection and GAB which are improved versions over previous MAS approaches, will help in improving abiotic stress tolerance by identifying and pooling several minor effect genes into the single line or genotype, further these techniques coupled with phonemics and latest speed breeding methods can fast track the process of cultivar development to better suit the cultivation environments during this changing climate scenario.

6.5 Diversity Analysis in Pigeonpea

Pigeonpea is the most versatile grain legume crop of arid and semiarid tropics of Asia, Oceania, Africa and America. A comprehensive knowledge on genetic relationship among species and extent of genetic variability provides valuable information on target trait availability and diversity for successful breeding programs.

6.5.1 Phenotype-Based Diversity Analysis

Increased use of available genetic resources/variability is a prerequisite for diversifying cultivar genetic base, enhancing current level of productivity and for continued genetic improvement to address biotic and abiotic stresses. Exploitation of natural genetic variability help to meet short-term objectives as very often breeders are forced to meet immediate requirement of the farmers, consumers and end-users. However, continued crop genetic improvement to meet medium- and/long-term requirements requires availability of variability induced through deliberately planned crosses among the genotypes harbouring desired combination of traits. The summaries

of variability studies related to different pigeonpea abiotic stresses are presented Table 6.5.

6.5.2 Genotype-Based Diversity Analysis

Genetic variability per se is of less significance in crop breeding programs. The knowledge on relative contribution of genetic and non-genetic sources on the quantitative trait variability is valuable in formulating appropriate selection strategies to breed improved cultivars. Several DNA-based markers are available for genetic diversity analysis and for various applications in plant breeding research as they are codominant, multi-allelic, highly polymorphic even between closely related lines, require low quantity of DNA, amenable for automated high throughput genotyping, highly transferable between laboratories and as well as among populations. The summaries of molecular marker studies pertaining to different pigeonpea abiotic stresses are presented Table 6.6.

6.5.3 Relationship with Other Cultivated and Wild Species

Wild relatives are crucial reservoirs of natural diversity due aboundence in biotic and abiotic stress tolerant genes. Despite rich genetic diversity created out of natural selection and domestication, loss of genetic diversity was reported in pigeonpea (Kassa et al. 2012a, b). The genus *Cajanus* is composed of 32–34 taxa which in turn divided into three clades viz., Indian, Australian, and Scarabaeoides. *Cajanus cajanifolius* (Haines) Maesen is the primitive in nature (Kassa et al. 2012a, b). Different species of pigeonpea (cultivated and wild) are listed here and the crossability aspects are detailed under Sect. 6.3. Despite species richness, molecular evidence suggests very low genetic diversity within cultivated pigeonpea (Saxena 2008) and envisages scope of pre breeding/wild gene introgression for crop improvement.

6.5.4 Relationship with Geographical Distribution

Kassa et al. (2012a, b) studied cultivated and wild relatives population structure and genetic diversity and identified three geographically distinct clades viz., domesticated and wild India clade, wild Australian clade and scarabaeoides clade. Further, multiple lines of evidence suggest recent gene flow between cultivated and non-cultivated forms, in addition to historical gene flow between diverged but sympatric species (Kassa et al. 2012a, b) and primary domestication occurred in India. Colin et al. (2015) studied global distribution of pigeonpea wild species and identified potential sites for their collection and conservation, and the same is presented in Table 6.7.

Table 6.5 Summaries of variability studies for different abiotic stresses in pigeonpea

Type of stress	Experiment material	Result/inference	References
Drought	10 cultivars of pigeonpea	Reported high variability for yield under stress and identified LRG 30, ICPL 85,063, and ICPL 332 as drought tolerant based on RWC, pods/plant and harvest index	Reddy (2001)
	Pigeonpea germplasm	Identified ICP 4575 as drought tolerant based on stress yield	Basu et al. (2016)
	Pigeonpea germplasm	Reported significant variation for physio-biochemical traits under water stress and identified RWC as the key trait for selecting tolerant genotypes	Kumar et al. (2011)
	26 extra-short duration pigeonpea genotypes	Under drought stress, there was significant genotypic variation in the onset, duration, and degree of osmotic adjustment (OA)	Subbarao et al. (2000)
	22 pigeonpea accessions	Studied variation for osmotic adjustment and dehydration tolerance of leaves and reported moderate variation among genotypes	Flower and Ludlow (1987)
	4 cultivars of pigeonpea	Under water stress, reported genetic variation in vegetative development, leaf water potential, RWC, photosynthesis, and stomatal conductance. Selection based on RWC was also suggested for identifying drought-tolerant pigeonpea genotypes	Kimani et al. (1994)
	9 short-duration pigeonpea genotypes	Drought resistance in pigeonpea was identified through genotypic differences, and selection	Lopez et al. (1996)

(continued)

Table 6.5 (continued)

Type of stress	Experiment material	Result/inference	References
Water-logging	Generations derived from two crosses (MA 98 PTH 1 × ICPL 84,023 and DA 11 × ICPL 84,023)	Observed that tolerance to WL in pigeonpea is governed by single dominant gene	Sarode et al. (2007)
	Short duration pigeonpea varieties	Reported that extra-early and early duration pigeonpea genotypes are more prone to WL compared to medium and long duration varieties	Matsunanga et al. (1991)
	272 diverse pigeonpea accessions	After three levels of testing, the following accessions were identified as tolerant: ICPH 2431, ICPH 2740, ICPH 2671, ICPH 4187, MAL 9, LRG 30, Maruti, ICPL 20,128, Asha, and MAL 15. (in vitro, in pots and in the field)	Sultana et al. (2013a, b)
Cold	Pigeonpea germplasm	Under cold stress, genetic variation for germinability and root length has been discovered	Kumar and Shukla (1991)
	Pigeonpea germplasm	Under cold stress conditions, there was a huge variation in plant mortality and survival	Yong et al. (2002)
	480 pigeonpea lines	Provided conclusive evidence for genetic variability for cold stress and identified 32 tolerant genotypes	Sandhu et al. (2007)
	Pigeonpea germplasm collected from different elevation zones of regions in Kenya	Under cold stress, there was a lot of variation in flowering habit, shelling percentage, and seed size	Upadhyaya et al. (2007)
	Pigeonpea germplasm	Low temperature effects on floral buds and flower drop were reported and seven highly tolerant genotypes, including Bahar, were identified. Long duration cultivars are also well adapted to cold stress	Singh et al. (1997)

(continued)

Table 6.5 (continued)

Type of stress	Experiment material	Result/inference	References
	Pigeonpea germplasm	Reported variability for buds/plant and flowers/plant under cold stress and identified IPA 7–2 as cold tolerant	Choudhary (2007)
Salinity[b]	Ten varieties of pigeonpea	Salinity stress had little effect on germination percentage and white seeded varieties are more salt tolerant than red or black seeded varieties	Karajol and Naik (2011)
	Cultivated and wild pigeonpea germplasm	Among cultivated genotypes and wild species, there has been reported genetic variation for survival under salt stress (Cajanus scarabaeoides)	Rao et al. (1981)
	Pigeonpea germplasm and its wild relatives	The extent of variation among cultivated pigeonpea genotypes is reported to be too small to warrant genetic salinity tolerance enhancement. At salinity levels of 8 dS m1 or higher, none of the pigeonpea genotypes tested survived longer than 30 days	Subbarao et al. (1991)
	6 genotypes of pigeonpea	There was no evidence of a link between early growth stages and adult salt tolerance. ICPL 151 was identified as salt tolerant because it accumulated significantly less Na+ and CI in salt-stressed shoots	Ashraf (1994)
Al toxicity	32 genotypes of Pigeonpea	Under the influence of aluminium toxicity, root growth was restricted and no significant change on the shoots were observed. At higher levels of aluminium, shorter roots with no normal branching pattern were observed	Singh and Choudhary (2009)

[b]Wild relatives of pigeonpea such as *C. scarabaeoides*, *C. albicans* and *C. platycarpus* showed varied level of salinity tolerance. In *C. albicans* salinity tolerance is controlled by dominant genetic trait hence, it is feasable to transfer to *C. cajan* (Choudary et al. 2011)

Table 6.6 Summary of pigeonpea molecular marker variability studies for various abiotic stresses

Type of material	Type of marker	Inference	References
All cultivated pigeonpea varieties of Malawi	48 polymorphic SSR	There was a lot of variation in the collection, according to the reports	Vincent et al. (2016)
16 cultivars of pigeonpea and two wild relatives (*C. albicans* and *C. lineatus*)	151 RAPD	Cultivars have a low level of genetic diversity, while cultivars and wild relative's posses a high diversity	Kusum et al. (2012)
47 domesticated pigeonpea germplasm	Sequence-specific amplified polymorphism (SSAP) and Retrotransposon-microsatellite amplified polymorphism (REMAP)	REMAP was proposed as a better alternative for distinguishing low genetic base pigeonpea genotypes	Maneesha (2017)
40 pigeonpea genotypes	54 SSR	Varieties were divided into seven major clusters in this study, each with a reasonable amount of variation	Romi et al. (2015)
12 diverse pigeonpea germplasm (8 wild and 4 cultivated)	40 RAPD and 40 SSR	RAPD markers are as effective as SSRs in analysing pigeonpea genetic diversity	Babasaheb et al. (2013)
Mutant population of pigeonpea	2 RAPD	The use of x-rays and gamma rays to induce mutations was found to be effective in increasing genetic diversity and RAPD was found to be effective in quantifying genetic variability	Khoiriyah et al. (2018)

(continued)

Table 6.6 (continued)

Type of material	Type of marker	Inference	References
77 landraces and 3 breeding lines from Benin	30 SSRs and 794 GBS derived SNPs	Both markers were found to be useful in polymorphism analysis, revealing a high level of genetic variability	Zavinon et al. (2020)
32 cultivated and 8 wild pigeonpea	36 SSR	A total of 72 alleles were discovered and phonetic analysis was used to distinguish between wild and cultivated genotypes	Saxena et al. (2010a, b)
88 pigeonpea accessions from India and East Africa	6 SSR	In comparison to East Africa, India had the most genetic diversity in terms of alleles, rare alleles, and Nei's unbiased estimate of gene diversity (H)	Songok et al. (2010)
15 cultivated and 9 wild pigeonpea accessions	39 SSR	Nineteen of the primer pairs were polymorphic among 15 cultivated and nine wild pigeonpea accessions, indicating that the genus Cajanus has cross-species transferability. All wild relatives were easily distinguished from each other and from cultivated germplasm using the diversity analysis	Odeny et al. (2007a, b)

(continued)

Table 6.6 (continued)

Type of material	Type of marker	Inference	References
96 accessions representing nearly 20 species of *Cajanus*	700 DArT	The majority of the diversity was found among pigeonpea's wild relatives or between wild and cultivated species. Genetic diversity among cultivated accessions is limited	Yang et al. (2006)
16 cultivars of pigeonpea and its two wild relatives (*C. albicans* and *C. lineatus*)	22 RAPDs and 10 ISSRs	Both the markers and the combined data revealed a similar pattern of narrow cultivar diversity and higher cultivar-to-wild diversity. When compared to RAPD and pooled data, the genetic diversity range obtained by ISSR markers was relatively higher	Yadav et al. (2014)
Cultivated and wild relatives (*Cajanus volubilis* and *Rhynchosia bracteata)* of pigeonpea	AFLP	Reported Pigeonpea cultivars have low polymorphism, but cultivated pigeonpea and its wild relatives have very high polymorphism	Panguluri et al. (2006a)

6.5.5 *Extent of Genetic Diversity*

Phenotype and DNA marker-based diversity studies of *Cajanus* revealed lack of genetic diversity in the primary gene pool. At the same time, wild Cajanus species serve as reservoirs for beneficial genes that can be used to improve GP1 and develop new climate-resilient cultivars (Rao et al. 2003; Sharma and Upadhyaya 2016a, b; Sharma et al. 2019). Despite high genetic diversity in the wild relatives, its use has been limited as no proper information on the presence of useful traits is easily available and an extended period of research is needed whenever utilized (Saxena

Table 6.7 Global distribution of pigeonpea wild species

Taxon	Countries identified for potential germplasm collection
C. acutifolius	Australia
C. cinereus	
C. confertiflorus	
C. lanceolatus	
C. latisepalus	
C. reticulatus	
C. scarabaeoides	Australia, Bangladesh, Bhutan, China, Fiji, India, Indonesia, Japan, Madagascar, Malaysia, Myanmar, Nepal, Papua New Guinea, Philippines, Sri Lanka, Taiwan, Thailand, Timor-Leste, Vietnam
C. crassus	China, India, Indonesia, Lao PDR, Malaysia, Papua New Guinea, Philippines, Thailand, Timor-Leste, Vietnam
C. cajanifolius	India
C. platycarpus	
C. sericeus	
C. albicans	India, Sri Lanka
C. lineatus	
C. trinervius	
C. mollis	Nepal

et al. 2014). By breaking the existing yield plateau, the introduction of beneficial genes from wild Cajanus may help to improve yield levels.

6.6 Molecular Mapping of Resistance Genes and QTLs

6.6.1 Brief History of Mapping Efforts in Pigeonpea

Genetic mapping is an important pre-requisite for the detection of molecular markers associated to traits of interest. In pigeonpea, due to narrow genetic variation in *Cajanus* primary gene pool and limited genomic resources viz, molecular markers, genetic linkage mapping has been challenging until 2010. Thereafter, a significant progress has been achieved in large scale development of molecular markers like SSR, DArT and SNP markers which facilitated construction of genetic maps, QTLs detections, candidate genes identification and genomics assisted crop improvement (Varshney et al. 2012a, b; Bohra et al. 2020; Saxena et al. 2020b). Exisiting markers systme such as SSRs, DArTs and SNPs tried in construction of genetic maps in pigeonpea (Sect. 6.4). Later, the interspecific mapping population (ICP 28 × ICPW 94) was used for genotyping with pigeonpea Kompetitive allele-specific polymerase chain reaction (KASP) assay markers (PKAMs) and developed a first high-density

genetic linkage map consisting 875 SNPs with a map length of 967.03 cM (Saxena et al. 2012). In another study, Kumawat et al. (2012) developed genetic map using inter specific cross Pusa Dwarf × HDM04-1. This map comprised 296 loci (267 SNPs + 29 SSRs) and covered a total map distance of 1520.22 cM. With the advancements in de novo discovery and high-density genotyping platforms genetic map resolution was enahnced significantly. More number of GBS-based high-density genetic maps were developed by Saxena et al. (2017b, c) (Table 6.8). Further, Arora et al. (2017) developed a consensus map based on three F_2 populations (Asha × UPAS 120, Pusa Dwarf × H2001-4 and Pusa Dwarf × HDM04-1). Very recently, Saxena et al. (2020a) genotyped the F_2 population comprising of 369 F_2 derived from the cross ICPA 2039 × ICPL 87,119 with the help of Axiom Cajanus SNP array with 56 K SNPs and generated a high-resolution map with 4867 SNPs that spanned 1580.68 cM.

6.6.2 Evolution of Marker Types

Molecular markers have been valuable and remain indispensable for molecular breeding and enhancing genetic gain in a short period. In pigeonpea, a wide range of molecular markers have been employed. Initially, several studies utilized first developed molecular marker systems such as restriction fragment length polymorphism (RFLP) (Nadimpalli et al. 1993; Sivaramakrishnan et al. 1997, 2002; Lakshmi et al. 2000), RAPD (Ratnaparkhe et al. 1995; Lohithaswa et al. 2003; Choudhury et al. 2008; Malviya and Yadav 2010) and amplified fragment length polymorphism (AFLP) (Panguluri et al. 2005; Wasike et al. 2005; Aruna et al. 2008) and applied mostly for estimation of genetic diversity and trait specific mapping in pigeonpea. Later, second generation markers such as SSRs became the marker of choice due to their abundance in the genome, codominant nature and multi-allelic nature and ease of scoring. SSRs developed from genomic libraries (Burns et al. 2001; Saxena et al. 2010a), mining of in silico based ESTs-SSRs (Dutta et al. 2011; Dubey et al. 2011a, b) and BAC-end sequences (BES-SSRs) (Aruna et al. 2008; Singh et al. 2008; Saxena et al. 2010b; Songok et al. 2010; Upadhyaya et al. 2011a, b). Bohra et al. (2011a, b) developed first large-scale SSR markers (3,072 SSRs) by extensive survey of BESs. Most of the SSRs were used for trait mapping and diversity analysis in pigeonpea. Later, with the advancement in NGS technology, third-generation marker systems with high throughput, more efficient and cost-effective nature came into existence. To this end, DArT arrays comprising a total of 15,360 features were developed (Yang et al. 2006). In addition, Saxena et al. (2011) identified a total of 5,692 SFPs. Further, Kudapa et al. (2012) developed markers of intron spanning region (ISR) from the transcriptome assembly. Subsequent development of efficient genotyping method by BeadXpress (Roorkiwal et al. 2013) and GoldenGate assay (Kassa et al. 2012a, b) facilitated high throughput SNP genotyping in pigeonpea. Further, a cost-effective SNP genotyping technology, KASP assay referred as pigeonpea KASP markers were employed to genotype 1,616 SNPs (Saxena et al. 2012). Subsequently, genotyping methods like GBS having ease in library preparation and higher

Table 6.8 The following is a list of pigeonpea genetic linkage maps that have been developed

Mapping population	Population size and type	No. of loci mapped	Marker system	Inter-marker distance (cM)	Total map length (cM)	Reference
ICP 28 × ICPW 94	F_2 (79)	239	BEC-SSRs	3.8 cM	930.90 cM	Bohra et al. (2011a, b)
ICP 28 × ICPW 94	F_2 (79)	122-Maternal	DArT	2.2 cM	270 cM	Yang et al. (2011)
ICP 28 × ICPW 94	F_2 (79)	172-Paternal	DArT	2.6 cM	451.6 cM	Yang et al. (2011)
ICP 8863 × ICPL 20,097	F_2 (190)	120	SSR	4.45 cM	534.89 cM	Gnanesh et al. (2011a, b)
TTB 7 × ICP 7035	F_2 (130)	78	SSR	5.98 cM	466.97 cM	
ICP 8863 × ICPL 20,097	F_2 (190)	120	SSR	4.5 cM	534.9	Bohra et al. (2012)
ICPA 2043 × ICPR 3467	F_2 (188)	140	SSR	6.3 cM	881.6	
ICPA 2043 × ICPR 2671	F_2 (188)	111	SSR	6.1 cM	678	
ICPA 2039 × ICPR 2447	F_2 (188)	78	SSR	7.3 cM	570.5	
TTB 7 × ICP 7035	F_2 (130)	78	SSR	6.0 cM	467	
ICPB 2049 × ICPL 99,050	F_2 (188)	59	SSR	9.9 cM	586	
Consensus map	Based on six mapping populations	331	SSR	3.1 cM	1059	
ICP 28 × ICPW 94	F_2 (167)	875	SNPs(PKAM)	1.11 cM	967.03	Saxena et al. (2012)

(continued)

Table 6.8 (continued)

Mapping population	Population size and type	No. of loci mapped	Marker system	Inter-marker distance (cM)	Total map length (cM)	Reference
Pusa Dwarf x HDM04-1	$F_{2:3}$ (183)	296	SNP + SSR	4.95 cM	1520.22	Kumawat et al. (2012)
ICPL 20,096 × ICPL 332	RIL (188)	1101	GBS-SNPs	0.84 cM	921.21	Saxena et al. (2017a)
ICPL 20,097 × ICP 8863	RIL (188)	484	GBS-SNPs	1.65 cM	798.25	
ICP 8863 × ICPL 87,119	F_2 (168)	996	GBS-SNPs	1.60 cM	1597.3	
ICPB 2049 × ICPL 99,050	RIL (188)	964	GBS-SNPs	1.45 cM	1120.56	Saxena et al. (2017b)
ICPL 20,096 × ICPL 332	RIL (188)	1101	GBS-SNPs	1.50 cM	921.2	
ICPL 85,063 × ICPL 87,119	F_2 (168)	557	GBS-SNPs	5.73 cM	1446.5	
ICP 5529 × ICP 11,605	F_2 (188)	787	GBS-SNPs	0.54 cM	1454.1	Saxena et al. (2017c)
Asha × UPAS 120	F_2 (92)	725	GBS-RAD SNPs	1.39 cM	1105.5 cM	Arora et al. (2017)
Pusa Dwarf × H2001-4	F_2 (94)	136	GoldenGate-SNPs	8.05 cM	943.58 cM	
Pusa Dwarf × HDM04-1	F_2 (183)	291	SNP + SSR	2.93 cM	930.3 cM	
Consensus map	Based on three mapping populations	932	SNP + SSR	1.51 cM	1411.83 cM	
ICPA 2039 × ICPL 87,119	F_2 (186)	306	GBS-SNPs	0.3 cM	981.9 cM	Saxena et al. (2018b)
ICPL 99,010 × ICP 5529	RIL (72)	6818	Axiom Cajanus SNP Array	0.1 cM	974 cM	Yadav et al. (2019)

(continued)

Table 6.8 (continued)

Mapping population	Population size and type	No. of loci mapped	Marker system	Inter-marker distance (cM)	Total map length (cM)	Reference
ICPA 2039 × ICPL 87,119	F_2 (369)	4867	Axiom Cajanus SNP Array	0.32 cM	1580.68 cM	Saxena et al. (2020a)

multiplexing capacity have offered a greater promise to simultaneously discover and genotype thousands of SNPs for genomics application (Saxena et al. 2017a, b, c, 2018b). Availability of reference genome sequence of pigeonpea greatly facilitated approaches like WGRS/skim sequencing. Recently, Kumar et al. (2016) revealed genome-wide variants including 4.6 million SNPs and 0.7 million InDels along with large structural variations (SVs) like copy number variation (CNV = 2598) and presence/absence variation (PAV = 970) based on HapMap of pigeonpea developed from WGRS data of 20 *Cajanus* accessions. Further, based on WGRS of 292 pigeonpea accessions including landraces, elite breeding lines and wild accessions, Varshney et al. (2017) also reported large SVs (≥1000 bp) in breeding lines (282 CNVs, 35 PAVs), landraces (228 CNVs, 37 PAVs) and wild species accessions (173 CNV, 77 PAVs).

6.6.3 Mapping Populations Used

An appropriate mapping population segregating for the traits of interest is essential for molecular mapping of traits under consideration. Based on morphological diversity, good number of mapping population generated. The difficulty of screening for abiotic stress caused challenges in population development mapping for each identified stress. An interspecific F_2 mapping population from the cross IPC 28 × ICPW 94 was used as reference for constructing SSR based reference linkage map (Bohra et al. 2011a, b and subsequently SNP based high density map (Saxena et al. 2012). As part of PGI, a total of 25 different mapping populations (F_2, F_3, backcross populations) segregating for important traits generated (Varshney et al. 2012a, b). In addition, several other studies used F_2 and $F_{2:3}$ populations for targeting important traits such as FW, SMD resistance, plant type, fertility restoration and earliness (Kotresh et al. 2006; Ganapathy et al. 2009; Dhanasekar et al. 2010; Gnanesh et al. 2011a, b; Bohra et al. 2012; Kumawat et al. 2012). In recent years, with the availability of high-density markers assays, RIL populations have been used for constructing high resolution genetic maps and trait mapping (Yadav et al. 2019). In addition, a reverse genetics approach like targeting induced local lesions in genomes (TILLING) population was developed through chemical mutagenesis (ethyl methane sulfonate) of an elite pigeonpea cultivar 'Asha' (Varshney et al. 2010a, b, c, d). Presently,

novel breeding populations, i.e., nested association mapping (NAM) and multi-parent advanced generation intercross (MAGIC) populations are being developed in pigeonpeato improve genomic diversity for line development.

6.6.4 Association Mapping

Association mapping helps in identify specific genetic variants associated with phenotypes. It considers use of association panels with diverse genotypes (such as germplasm lines, wild relatives, landraces, elite cultivars, etc.) in order to record historic recombination events that contribute to dissection of complex phenotypes with increased allelic richness and higher mapping resolution (Ibrahim et al. 2020). QTL analysis in F_2 population (ICPA 2039 × ICPR 2447) revealed a candidate gene '*CcTFL1*' controlling determinacy in pigeonpea explaining phenotypic variation of 45–96% for determinacy and 77% for plant height. Recently, genome-wide association analysis using whole genome resequencing of 292 accessions comprising breeding lines, landraces and wild species of pigeonpea identified a 241 MTAs for agronomical traits (Varshney et al. 2017). Further, molecular marker such as SNPs with cost affective genotyping platforms and high throughout phenotyping technologies will encourage application of more association studies for germplasm enhancement in pigeonpea.

6.6.5 Trait Mapping

Initially, bulked segregant analysis (BSA) approach used in pigeonpea for fusarium wilt (FW) resistance (Table 6.9). For example, using F_2 based BSA approach; Kotresh et al. (2006) identified two RAPD markers (OPM03704 and OPAC1150) for FW resistance. Also, Dhanasekar et al. (2010) identified two RAPD markers ($OPF04_{700}$ and $OPA09_{1375}$) for plant type. Further, Daspute and Fakrudin (2015) detected a repulsive-phase RAPD marker co-segregating with SMD resistance. Recently, Khalekar et al. (2014) identified five SSR markers (PFW 26, PFW 31, PFW 38, PFW 56, and PFW 70) that can distinguish between with susceptible and resistant bulks of FW (Singh et al. 2016a, b). Resistant and susceptible bulks from two extreme RILs from ICPL 20,096 × ICPL 332 were sequenced and subsequently detected seven candidate SNPs for resistance.

QTL mapping was first carried out by Gnanesh et al. (2011a, b) using two $F_{2:3}$ families derived from the crosses ICP 8863 × ICPL 20,097 and TTB 7 × ICP 7035 and identified six QTLs (qSMD1 to qSMD6) controlling sterility mosaic disease (SMD) resistance having phenotypic variation ranging between 8.3–24.7% and 10.58–24.72%, respectively (Table 6.9). Similarly, Bohra et al. (2012) discovered four major QTLs (Phenotypic variation explained (PVE) = 14.85–24.17%)

Table 6.9 Tolerance mechanism for abiotic stress idenetified in differnt genotypes of pigeonpea germplasm by Choudhary et al. (2013)

Abiotic stress		Tolerance mechanism and cultivars in which identified
Moisture stress	Waterlogging	Development of lenticels, increased root biomass, and adventitious root in Asha (medium), ICPL 84,023 (early)
	Drought	RWC, pods/plant, and HI all contribute to a high RWC in LRG 30, ICPL 332 (medium), ICPL 85,063
Temperature stress	Low temperature	The ability to flower and set pods at low temperatures in IPA 7–2, MAL 19 (Late) and Bahar
Mineral stress	Salinity	Na and Cl translocation from the root to the shoot is reduced in C11, ICPL 227, WRP1, GS1 and TS3 (medium) UPAS 120 and ICPL 151 (early)
	Aluminium toxicity	Exclusion of aluminium in IPA 7–10 and T 7 (late), GT 101E (early) and 67 B

for fertility restoration based on the genotyping and phenotyping data of three F_2 populations.

6.6.6 Next-Generation Based Trait Mapping

The available information on draft genome sequence, resequencing data (Kumar et al. 2016) and development of next-generation sequencing (NGS) technologies such as genotyping-by-sequencing has enabled high-resolution trait mapping of economically important traits using intra-specific mapping populations of pigeonpea. For instance, Saxena et al. (2017b) used GBS approach to discover SNPs and genotype in two RIL populations (ICPB 2049 × ICPL 99,050 and ICPL 20,096 × ICPL 332) and a F_2 population (ICPL 85,063 × ICPL 87,119). QTL analysis based on GBS data and varied location and year phenotyping data of fusarium wilt revealed 14 significant QTLs for FW resistance with PVE ranged between 2.65–56.45% (Table 6.9). Further, using GBS approach, QTL for growth habit locus (*Dt1*) contributing more than 61% PVE was mapped on CcLG03 of the pigeonpea genetic map in a F_2 population of (Saxena et al. 2017c). QTL analysis using genotyping and phenotyping data identified 10 QTLs for SMD resistance with PVE ranging from 3.6–34.3%. Saxena et al. (2020c) used two backcross populations developed through interspecific crosses (ICPL 87,119 × ICPW 15,613 and ICPL 87,119 × ICPW 29) and discovered a total of 86 (PVE = 12–21%) and 107 QTLs (PVE = 11–29%) respectively, associated with nine yield related traits. Yadav et al. (2019) genotyped the RIL population and detected five QTLs (PVE = 9.1–50.6%) for cleistogamous flower, three QTLs (PVE = 11.8–37.2%) for shrivelled seed and one QTL (PVE = 29.5%) associated with seed size. Similarly, Saxena et al. (2020a) also constructed a high-resolution genetic

map based on Axiom Cajanus SNP Array in a F_2 generation and detected four QTLs (PVE = 2.34–45.06%) for *Rf*. Recently in pigeonpea, further advances in development of trait specific markers lead to development of a diagnostic kit with 10 markers each for identification of FW and SMD resistant lines (Saxena et al. 2021b).

6.7 Marker-Assisted Breeding for Resistance Traits

DNA markers are important genomic tools to study the germplasm, varietal identification, genetic diversity, linkage map, gene tagging, marker assisted selection and association mapping studies. Pigeonpea genomic initiative has focused mainly on the development of set of molecular markers including microsatellites, SNPs and diversity array technology markers (Gupta and Varshney 2000) helped in crop improvement for both biotic and abiotic stress.

6.7.1 Germplasm Characterization and DUS

Variability for pigeonpea germplasm is found in the different states of India and these regions show tremendous variations for both domesticated and wild relatives belong to GP1 and GP2 (Smartt 1990). Studies showed considerable variation for morphological, reproductive, nutrient substance for stress tolerance related traits. Biochemical markers engaged to detect polymorphism in the genus *Cajanus.* Study on esterase isozymes showed species affinity between pigeonpea and some of its wild relatives, revealing the relationships between *C. scarabaeoides, C. albicans, C. sericeus and C. volubilis* (Krishna and Reddy 1982). Ratnaparkhe et al.(1995) explained two closely related *Cajanus* species, (*C. scarabaeoides and C. cajanifolius*) showed a close association with one another, Upadhyaya et al. (2011a, b) use the set of SSR markers to separate wild and cultivated types in two classes based on their allelic variation. AFLP analysis on cultivated pigeonpea showed genetic similarity among genotypes (Panguluri et al. 2006b); a supportive study on SSR-based analysis conducted by Odeny et al. (2007a, b) showed that the cultivated species was less polymorphic than the wild relatives.

Germplasm accessions in pigeonpea have been evaluated using trait specific descriptors (Remanandan et al. 1988). A majority of pigeonpea germplasm accessions have been evaluated for different morphological, agrinomical, biotic and abiotic stresseses (Table 6.9). The significant work at ICRISAT and other leading institutes has paved the path for identification of genes tolerance to soil salinity and drought (Kooner and Cheemar 2006 and Reddy et al. 1981). The pigeonpea core collection, comprising of 1290 accessions sampled from 12,153 germplasm accessions from 53 countries, was developed at ICRISAT (Frankel 1984). Recently, the draft sequence of pigeonpea and annotated 48,680 genes and their potential role in unravelling drought tolerance has been reported by Varshney et al. (2012a, b).

6.7.2 Marker-Assisted Gene Introgression

Drought tolerant progenies ($BC_3F_{3:4}$) in the genetic background of 'JG 11' (a popular Indian chickpea cultivar) have been developed by the transfer of a genomic region, a "QTL hotspot" from the donor 'ICC 4958' that carries several QTL for drought tolerance. Initiatives have also been under taken to use marker-assisted recurrent selection (MARS) in chickpea to develop and identify drought-tolerant lines having favourable alleles using the crosses ICCV04112 × ICCV93954 and ICCV05107 × ICCV94954 (Dua et al. 1996). In pigeonpea, few candidate genes such as *CcHyPRP, CcCYP*, and *CcCDR* genes that control drought, salinity and cold is identified and validated. Recently, the Al-responsive *CcSTOP1* and *CcMATE1* genes analyized (Abhijit et al. 2017) this will contribute to development of soil acidity tolerance genotypes. Marker assisted introgression studies were carried to transfer drought tolerance from ICC 4958 to JG 11 and from ICC 8261 to two kabuli chickpea cultivars 'KAK 2' and 'Chefe' (Gaur et al. 2012). With the similar goal of introgressing drought tolerance, substantial use of MABC was demonstrated to be effective in transferring a QTL-hot spot from ICC 4958 harbouring many QTLs realted to root and drought traits to a popular high-yielding cultivar 'JG11' (Varshney et al. 2013a). Besides MABC, MARS propounded, which are able to tap the genetic variation that is accounted to smaller effects QTLs (Varshney et al. 2013c).

6.7.3 Gene Pyramiding

Gene pyramiding is a crop breeding technique that can be applied in conventional and advanced molecular breeding programs to introduce novel lines. Gene stacking or pyramiding is a useful technique for transferring several desired genes or QTLs from different parents into a single genotype in the shortest possible time (two to three generations), as compared to conventional method which need minimum of 6 generation to recover 99.2% of recurrent parent genome (Suresh and Malathi 2013). It aims at accumulating several resistance genes with known effect on a trait of target and confers durable resistance against different stress (Das et al. 2017).

Genetic maps and molecular markers can help in the identification of rare recombinant events leading to the breakage of linkage thus reducing the amount of deleterious gene combination in the new genetic background. One of the modern breeding approaches used for systematic introgression of donor genes is MABC in gene pyramiding approach. MABC relies on the precise donor genomic fraction selection through tightly linked or flanking markers to the target locus/QTL (foreground selection) (Varshney et al. 2010c). Nevertheless, in contrast to MABC, which exploits pre-estimated QTL effects, MARS scheme involves the construction of an ad hoc marker index, and this is further accompanied by marker indexbased selections of desirable genotypes and intercrossing of the selected individuals in advanced generations. MARS was attempted recently in chickpea (Gaur et al. 2012). Similar to

MARS, advanced backcross QTL (AB-QTL) is another molecular breeding scheme that does not need predefined gene-trait associations (Tanksley and Nelson 1996). Given its ability to capture the tremendous genetic variation present in AB-QTL scheme tried in chickpea at ICRISAT (Gaur et al. 2012, Varshney et al. 2013b). Mapping of important traits will facilitate identification of tightly linked markers for marker-assisted selection. Marker trait/QTL association studies and mining rare alleles will help in cross species transfer and development of tolerant genotypes in abiotic stress in pulse breeding. Development of MAGIC & NAM populations which have been popularized for their capability in utilizing huge data on various loci for genome assisted breeding involving multiple parents apart from biparental crosses trait mapping will be the potential breeding strategy for increasing productivity.

6.7.4 Limitations and Prospects of MAS and MABCB

Genetic maps and molecular markers can help in the identification of rare recombinant events leading to the breakage of linkage thus reducing the amount of deleterious combination of genes in the new genetic background. One of the recent breeding approaches used for systematic introgression of donor genome is MABC. Prerequisite for MABC is the tight association between QTL of interest and moecular marker and it depends on the foreground selection; accompanied with a background selectionto maximize the recovery of recurrent parent genome (Varshney et al. 2010c).

Abiotic stress tolerance improvement both in chickpea and pigeonpea by involving GP2 and GP3 wild species is hindered by cross incompatibility barriers, F_1 sterility, linkage drag and different phonologies of both cultivated and wild species (Berger et al. 2005). Some of these hindrances can be overcome through exploitation of special techniques such as application of growth hormones followed by ovule culture and embryo rescue (Mallikarjuna and Jadhav 2008). For transfer of superior alleles from wild species (salinity tolerance in *C. albicans*), an AB-QTL approach (Tanksley and Nelson 1996) may be used, as it facilitates efficient tracking of desired and non-desired alleles. MABC using markers-trait linked may also be used to develop superior lines or QTL is identified and validated in the donor, as it will facilitate retaining the whole genome of their current parent (Hospital 2003). Nevertheless, root traits, drought tolerance score, canopy temperature differential seed size in chickpea are governed by many QTL. Under such a situation, MARS, which involves inter crossing among selected individuals in each cycle of selection, may be used to avoid the limitations of MABC (Choudhary et al. 2013).

Genomic selection through genome sequencing approach and NGS technology help in exploring nucleotide level diversity and help in overcoming difficulties for breeding for quantitative trait associated with low heritability through MABC/MARS. The advanced genomic technologies like breeding values estimation based on parameters called genomic-estimated breeding values (GEBVs) and

MOOB assist genetic gains by combing useful gene combination and enhancing multi trait selection for desirable genotype.

6.8 Map-Based Cloning of Resistance Genes

DNA libraries are useful in mapping, cloning and sequencing in higher eukaryotes (Hosoda et al. 1990 and Zhang et al. 1996). Because of its ability to maintain large DNA fragments and ease of manipulation, BAC cloning has become an invaluable tool in genomic studies (Wang et al. 1995). BAC libraries are a valuable resource for the development of molecular markers for MAS of desirable agronomic traits. SSR markers made from BAC-end sequences are inexpensive and provide genome-wide coverage because all repeat types are systematically sampled in the randomly selected BACs (Shultz 2007b; Cho et al. 2004).

6.8.1 Traits and Genes Targeted for Map-Based Cloning

Ta96 is a sequence tagged microsatellite site (STMS) marker closely associated (1 cM) to FW resistance gene (*Foc3*). This marker was used to screen the BAC library, which was constructed to detect FW resistance in the germplasm line namely FLIP 84-92C (Rajesh et al. 2004). In another study the Ta96 mapped to 2nd linkage group where other wilt resistance (R) genes of the same pathogen were located (Ribaut 2008). Lichtenzveig et al. (2005), developed BAC as well as BIBAC (plant transformation-competent binary BAC) library for chickpea cv. Hadas. The abundant SSRs present in chickpea have a high level of polymorphism, according to Winter et al. (2000), which make them to useful for mapping and gene tagging.

6.8.2 BAC Library for Cloning

BAC library is an important resource for the development of molecular markers that can be used for MAS for desirable agronomic traits. Development of SSR markers from BAC-end sequences is very cost effective (Temnykh et al. 2001) and offers genome-wide coverage as all repeat types were systematically sampled in the randomly selected BACs. Varshney et al. (2010a, b, c, d), end-sequenced 50,000 randomly selected clones from this BAC library generating a total of 87,590 BESs. These were screened with a microsatellite search module resulting in the identification of 18,149 SSRs representing 6,590 BAC clones.

Researchers at UC Davis discovered 756 BAC clones that could form the base for an SSR molecular resource linked to 90 BAC contigs using NBS-LRR (nucleotide binding site leucine rich repeat disease resistance) homologues based on Medicago

truncatula (Varshney et al. 2010a, b, c, d). This information is useful in molecular breeding; the availability of a BAC library, in addition to high-density molecular maps, transcription based sequences information and other tools will revolutionise the improvement of pigeonpea crops.

6.8.3 Expression of Cloned Genes

Biotechnological approaches for pulse crop improvement, genetic transformation strategies have likewise been slow to be implemented. ICRISAT has taken a leading role in recent years at improving mandated pulse crops for abiotic stress tolerance, especially drought tolerance and include use of genetic transformation technology (Bhatnagar et al. 2010). Transgenic chickpea lines over-expressing a mutagenized pyrroline-5-carboxylate synthetase (*P5CS*) gene led to elevated proline levels under water deficit in the greenhouse, but effect on yield was nonsignificant was noticec, although transpiration efficiency was modestly improved (Bhatnagar et al. 2009). A similar strategy in soybean, but using the LΔ1-pyrroline-5-carboxylate reductase (*P5CR*) gene showed elevated accumulation of proline under stress and improved the ability to metabolize proline after re-watering (De ronde et al. 2004). Interestingly, a *P5CS* gene from *Vigna aconitifolia*, altered by site-directed mutagenesis to prevent feed back inhibition of proline (Zhang et al. 1995), was used to produce transgenic tobacco plants with increased drought tolerance (Gubis et al. 2007). Such studies are encouraging and especially since the *P5CS* gene was cloned from a pulse crop. The most extensively studied transcription factors were the dehydration responsive element-binding/C-repeat-binding (*DREB/CBF*) identified in Arabidopsis and its involvement in multiple abiotic stresses (Liu et al. 1998 and Jaglo et al. 1998).

6.9 Genomics-Aided Breeding for Resistance

6.9.1 Details of Genome Sequencing

The first draft genome sequence information on pigeonpea was published by Varshney et al. (2012b). The famous pigeonpea variety ICPL 87,119 (Asha) was used for the sequence project, using Illumina next-generation sequencing platform. The study estimated the pigeonpea genome size (833.07 Mb), based on K-mer statistics. Numbers of scaffolds reported were 137,542 among which 6,534 had scaffolds longer than 2 kb. They predicted about 48,680 genes through genome analysis. The group identified certain gene families, like drought tolerance–related genes that could have played a role in the domestication of pigeonpea and evolution of its ancestors. Similarly, Singh et al. (2012a, b) by using the same popular variety *"Asha"* published draft genome sequence information, which was previously used to by Varshney et al.

(2012b). The genome was assembled using 454 GS-FLX sequencing chemistry, which produced 510,809,477 bp (base pairs) of high-quality sequence with mean read lengths of > 550 bp and > tenfold genome coverage. A total of 47,004 protein-coding genes and 12,511 genes related to transposable elements were predicted. The sequence contigs were organized into 59,681 scaffolds that were anchored to eleven pigeonpea chromosomes with 347 genic-SNP markers from an intra-species genetic map. They also reported 1,213 disease resistance/defense response genes and 152 abiotic stress tolerance genes suggesting reason for the hardy nature of pigeonpea crop.

In order to detect and characterize genome-wide variation in pigeonpea, 292 *Cajanus* accessions including wild species, landraces, breeding lines were resequenced by Varshney et al. (2017). They studied patterns of variation across *Cajanus* accessions, phylogenetic relationships, impact of domestication, genetic diversity and genome-wide associations between candidate genes and agronomically important traits. Flowering time control, seed development and pod dehiscence were some of the traits for which candidate genes had sequence similarity to genes which were functionally characterized in other plants.

The genome sequences information will hasten the use of pigeonpea germplasm resources in breeding (Yang et al. 2006; Saxena 2008; Varshney et al. 2010a, b, c, d). Sequences generated will be helpful in developing markers (SSR and SNP) for diversity analysis in the germplasm and are also useful for fingerprinting, genetic mapping and trait identification. Molecular breeding approaches such as marker-assisted backcross selection, marker-assisted recurrent selection, association studies and genomic selection can be fallowed in pigeonpea.

6.9.2 Organelle Sequencing

6.9.2.1 Mitochondrial Genome Sequencing

Exploitation of heterosis in pigeonpea, require hybrid breeding technology. CMS possesses huge potential to develop high yielding pigeonpea hybrids. CMS is the result of the production of novel chimeric open reading frames (ORFs) due to rearrangements in the genomes of mitochondria. Recognition of these CMS-related ORFs in pigeonpea, Tuteja et al. (2013) sequenced following mitochondrial genomes viz., The male-sterile line of ICPA 2039, the maintenance line of ICPB 2039 and the hybrid line of *C. cajan* (ICPH 2433), as well as the wild relative of *Cajanus cajanifolius,* (ICPW 29). For the ICPA 2039 line, a single circular-mapping molecule with a length of 545.7 kb was assembled and annotated. Genes (51) were predicted using sequence annotation which included 34 protein-coding and 17 RNA genes. The mitochondrial genomes of different *Cajanus* genotypes were compared, and 31 ORFs were found to differ across lines in which CMS is present or absent. By comparing the related male-sterile and maintainer lines, 13 chimeric ORFs were discovered. These ORFs has been associated to trigger CMS in other plants.

6.9.2.2 Chloroplast Genome Sequence

The draft chloroplast genome of *C. scarabaeoides* and *C. cajan* were sequenced by Kaila et al. (2016) by using Roche 454 technology. *C. scarabaeoides* an important species in *Cajanus* gene pool, has been employed by many groups to construct promising CMS systems. The plastid genome used for sequencing was derived from a male sterile genotype with *C. scarabaeoides* cytoplasm. The reported chloroplast genome of *C. cajan* and *C. scarabaeoides* was 152,242 bp and 152,201 bp respectively, both having a quadripartite structure, further they also discovered 116 novel genes, including 78 predicted protein coding genes, and 5 pseudogenes, 4 rRNAs, 30 tRNAs. In the large single copy (LSC) region of the pigeonpea chloroplast genome, a 50 kb inversion was also observed as in other legumes.

6.9.2.3 Gene Annotation

Process of identifying functional elements along the sequence of a genome using bioinformatics tool can be called as gene annotation. To forecast gene models in the pigeonpea genome, Varshney et al. (2011) used combination of homology-based methods and de novo gene prediction programs and these were based on the GLEAN algorithm (Elsik et al. 2007). Singh et al. (2012a, b) have used FGENESH tool of MOLQUEST software (www.softberry.com) using *Arabidopsis thaliana* gene models as reference for gene annotation. Tuteja et al. (2013) predicted protein-coding and RNA genes by performing BLASTX and BLASTN searches respectively. Kaila et al. (2016) carried out genome annotation with DOGMA (Dual Organellar Genome Annotator; Wyman et al. 2004) to identify coding sequences (cds), rRNAs, and tRNAs using the plastid genetic code and BLAST homology searches.

6.9.3 Application of Genomics-Assisted Breeding

The publication of draught genomes in pigeonpea has paved the way for trait mapping and molecular breeding techniques based on re-sequencing (Singh et al. 2015) used these information to map a number of economically important traits, including FW and SMD, plant type and earliness (Kumawat et al. 2012), CMS (Sinha et al. 2015a, b, c) and hybrid purity estimation are examples of agronomic traits (Saxena et al. 2010a, b).

6.10 Recent Concepts and Strategies Developed

6.10.1 Gene Editing

Numerous new plant breeding techniques (NPBTs) have been developed over the last 20 years (Lusser and Cerezo 2012). NPBTs make specific changes to the plant's genetic blueprint in order to modify its targeted traits and these changes can range from minor adjustments or deletion of one or more genes. There are a variety of methods for achieving these changes, including processes that alter gene activity without altering the genetic blueprint (epigenetic methods), grafting of unaltered plant pieces onto a genetically modified rootstock, and genome editing (Dima et al. 2020). Mega nucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated systems (Cas) are all examples of site-directed nucleases (SDNs) that are currently available. Because of CRISPR/Cas technology, which was only introduced in 2013, but is now by far the most popular tool for creating targeted changes in the heritable part of the blueprint due to its simplicity (Kaul et al. 2020), genome editing technology has emerged as a multifaceted strategy that has instantaneously popularized the mechanism to change the genetic nature of an organism (Modrzejewski et al. 2019; Kaul et al. 2020).

GE has a plethora of tools at its disposal, highlighting their significant implications for crop improvement and the regulatory concerns surrounding their viability (Kaul et al. 2020). CRISPR/Cas9-based technique with non-homologous end-joining (NHEJ) and homology-directed repair (HDR)-mediated repair pathways could result in genome editing. As the cell ligates double-strand breaks (DSB) within DNA, random nucleotide insertions and/or deletions occur, resulting in gene disruption. The DSB is repaired in HDR by using an externally supplied homologous donor repair template, which results in directed precision repairing.

CRISPR/Cas9-based genome modification has aided crop breeding by allowing for targeted genome editing of a variety of agronomically important traits (Chen et al. 2019; Schindele et al. 2020). Editing a single gene, *ZmLG1*, resulted in erect architecture in corn, and these plants showed increased density in the field (Li et al. 2017; Tian et al. 2019); directed mutagenesis using CRISPR/Cas9 editing controlled the *FT2* gene, i.e., GmFT2a and GmFT5a, which regulated the photoperiod in soybean (Cai et al. 2018). Similarly, HDR-guided base transversions were used to create herbicide-resistant soybean plants (Li et al. 2015). A large number of genes with phenotypic significance have been identified to generate useful traits in crops such as rice, maize, wheat, sugarcane, soybean, potato, sorghum, orange, cucumber, tomato, flax, and cassava, for traits such as herbicide resistance, drought tolerance, thermosensitive genic male sterility, disease resistance, and altered product quality, with some already on the market (Zhang et al. 2018). Crop improvement for food and nutritional security, particularly in light of population growth and challenges such as climate change and water scarcity, have become important global concerns (Hickey et al. 2019).

Current crop breeding strategies, according to Varshney et al. (2020), will not yield an enough amount of crop development to meet demand in the short- or long-term future. As a consequence, a 5G breeding strategy was proposed to dramatically speed up crop genetic improvement. The first G stands for genome assembly for each crop species, the second G stands for genomic and germplasm characterization for agrnomic traits, the third G stands for gene function identification, the fourth G stands for genomic breeding methodologies, and the fifth G stands for gene editing technologies. In any breeding program, including in developing countries where these gains are most needed. A comprehensive application of 5G breeding can improve the precision, efficiency, and effectiveness of breeding programs to develop climate-resilient, high yielding, and nutritious varieties while delivering a high rate of genetic gain. The usage of genome editing technology in plants aids in the unravelling of basic biology facts, with the addition of genome-wide association studies, artificial intelligence and various bioinformatic frameworks; is resulting in futuristic model studies and their confirmation. The strategies for reducing "off-target" effects and gaining societal acceptance of genome-modified crops developed using this modern biotechnological approach have been examined (Kaul et al. 2020).

6.10.2 Nanotechnology

Plants respond to abiotic stress in a variety of ways, including changes in their morphology, composition and metabolism. A variety of strategies tried to improve abiotic stress tolerance, including the development of genetically engineered varieties containing various gene constructs that are thought to improve performance under stress. Nanotechnology is a multifaceted field that has applications in almost every field of science. Nanoparticles improved seed germination and growth of seedling, as well as physiological activities, nitrogen metabolism, chlorophyll content, protein, carbohydrate content, and yield, as well as positive changes in gene expression, indicating their potential for crop improvement (Das and Das 2019). Surabhi et al. (2021) investigated the impact of seed treatment with ZnO, Ag, and SiO_2 nanoparticles on seed quality and storage potential on seeds of pigeonpea. The effects of nanoparticles on seed quality and storability in pigeonpea were found to be significant. At 0, 6, and 10 months of storage, seeds treated with SiO_2 NPs @ 250 mg outperformed seeds treated with other treatment combinations in terms of germination, mean seedling dry weight, seedling vigour index-II, field emergence, total dehydrogenase activity, lower electrical conductivity, and reduced seed moisture content. These findings suggest the possibility of application of nanotechnology in enhancing seed quality and storability of pigeonpea.

The effect of seed polymer coating of nanoparticles (NPs) Zn, Fe at different concentrations (10 and 25 ppm), $ZnSo_4$, $FeSo_4$ (100 and 500 ppm), and hydro priming with different durations (6 and 12 h.) on seedling characters of pigeonpea seed was investigated in a laboratory setting. Seed polymer coating with Fe NPs at 25 ppm resulted in significantly higher seed germination, speed of germination, seedling

root length, seedling shoot length, seedling length, seedling dry weight, seedling fresh weight, seedling vigour index I, seedling vigour index II, and lowest abnormal seedlings when compared to their bulk forms and control, subsequently Fe and Zn NPs at 25 ppm. Individually, hydropriming had a positive effect on pegionpea seed quality parameters, but the effect of priming method was found to be significant. As a result of the findings, Fe and Zn NPs at 25 ppm can be used to improve the quality of pigeonpea seed (Raju and Rai 2017).

Raghu et al. (2017) investigated the impact of macro and nano insecticides on pigeonpea seed germination and vigour. Using a high-energy planetary ball mill, different recommended seed treatment insecticides such as malathion, fenvalerate, emamectine benzoate, thiodicard, sweet flag, and neem seed kernel powder insecticides were synthesized to nano form. Seed treated with nano malathion 50 percent less than normal dosage, fenvalerate 60% less, thiodicarb 10% less, emamectine benzoate 30% less, sweet flag 70% less, and neem seed kernel powder 40% less than actual recommended dosage had significantly higher seed germination, fewer abnormal seedlings, and shooting.

Throughout their lives, plants are subjected to a variety of environmental stresses, so they develop defence mechanisms at various levels by modulating molecular, biochemical, and physiological pathways. Plants adapt molecular routes to cope with these stresses by changing gene expressions appropriately. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) are all upregulated by nanoparticles (Laware and Raskar 2014). Nanotechnology promises to make a significant contribution to mitigating abiotic stresses. Several recent studies (Table 6.10) have looked at nanoparticle-mediated stress in various situations (Barrena et al. 2009; Lee et al. 2010). The uptake and translocation of NPs through substantial physical inertial barriers from the root surface to vessels of xylem, firing upward through the root surface cuticle, epidermis, cortex, endodermis, casparian strip and finally transports in shoot through the xylem (Lv et al. 2019) and number of factors influence on root during NPs absorption (Luque 2017).

Plant stress tolerance is improved by nanoparticles, which increase root hydraulic conductance and water uptake while also revealing differences in the abundance of proteins involved in oxidation–reduction reactions, ROS detoxification, stress signaling, and hormonal pathways. When nanoparticles interact with plant cells, they alter plant gene expression and biological pathways, affecting plant growth and development. Nanotechnology in agriculture research necessitates a thorough examination of fabrication, characterization, standardization, biodegradability, eco-friendliness, as well as the potential uptake and translocation of nanoparticles by plants (Das and Das 2019).

Table 6.10 Abiotic stress and its consequences in relation to nanomaterials in various crops

Abiotic stresses	Nano material	Concentration	Crop species	Stress responses	Refrences
Drought stress	Nano TiO_2	0.01, 0.02, and 0.03%	Wheat (*Triticum aestivum* L.)	Increasing growth, yield, gluten and starch content of wheat	Jaberzadeh et al. (2013)
	Nano ZnO	0.5, 1 g L^{-1}	Soybean (*Glycine max* L.)	Increasing the percentage of seeds that germinate and the rate at which they germinate, while lowering the seed residual fresh and dry weight of soybeans	Mahmoodzadeh et al. (2013)
	Silicon		Sorghum (*S bicolar*	Leaf area index (LAI), specific leaf weight (SLW), chlorophyll content (SPAD), leaf dry weight (LDW), shoot dry weight (SDW), root dry weight (RDW), and total dry weight (TDW) all increased (TDW)	Ahmed et al. (2011)
Salinity stress	Nano ZnO	2 g L^{-1}	Sunflower (*Helianthus annuus* L.)	In leaves; growth, net CO_2 assimilation rate, sub-stomatal CO_2 content, chlorophyll content, Fv/Fm and Zn content are all increasing, while Na+ content is decreasing	Torabian et al. (2016)
Salinity stress	ZnO	2 g L^{-1}	Sunflower (*Helianthus annuus* L.)	Increased growth, proline content, and the activity of some antioxidant enzymes	Torabian et al. (2016)
Flooding stress	Nano Al2O3		Soybean (*Glycine max* L.)	Improved growth and regulation of energy metabolism and cell death	Mustafa et al. (2015)

(continued)

Table 6.10 (continued)

Abiotic stresses	Nano material	Concentration	Crop species	Stress responses	Refrences
	Nano Ag		Soybean (*Glycine max* L.)	Reduced production of cytotoxic glycolysis byproducts, increased abundance of stress-related proteins, and improved seedling growth	

6.11 Genetic Engineering for Resistance

6.11.1 Transgenic Achievements in Pigeonpea

The transgenic knowledge has revealed remarkable achievements in crop protection by overcoming the major breeding barriers. These techniques provided a long-term support to the research efforts for incorporation of agronomically convenient traits, which have a positive impact on crop improvement and nutritional security of world-wide human population (Saxena et al. 2016a, b). The ability to introduce foreign genes through genetic transformation in host plant tissue is entirely relied on cellular totipotency to serve as the recipients for successful DNA delivery method (Atif et al. 2013). The accessibility of various transformation methods has made successful transgenic development in different crop species and among those *Agrobacterium tumefaciens*-mediated genetic transformation is convenient and frequently used technique on a wide array of plants.

Through genetic transformation technology, more than 15 genotypes of pigeonpea were used by researchers for the development of improved cultivars by extending the nutritional quality level or through incorporation of resistance/tolerance against several stress factors (Ghosh et al. 2014a). Among them ICPL87 was the most frequently used genotype with the achievement of 80% transformation frequency (Krishna et al. 2010). Over the last few decades, several attempts were made in pigeonpea to introduce different foreign genes through *Agrobacterium*-mediated transformation strategy but the success rate was immensely constricted by its poor tissue culture response (Ghosh et al. 2014a, b). Various genes like *Bacillus thuringiensis* endotoxins *cry1A (b), cry1Ab, cry1Aabc, cry1AcF, cry1Ac, cry2Aa, cry1 E-C* (Surekha et al. 2005; Sharma et al. 2006; Ramu et al. 2012; Das et al. 2016; Ghosh et al. 2017) and *cowpea protease inhibitor* (*CPI*) (Lawrence and Koundal 2001) were used to develop transgenic pigeonpea with a higher level of toxicity against the lepidopteran insects. Additionally, *rice chitinase* (*Rchit*) gene was also

introduced in this legume for improving the resistance level to fungal pathogen (Kumar et al. 2004). Apart from the above-mentioned reports, pigeonpea transformation was also conducted with different edible vaccine genes like *hemagglutinin* gene of render pest virus *(RPVH)* and *hemagglutinin neuraminidase* gene of peste des petits ruminants' virus *(PPRV-HN)* to improve the goat and sheep immune system against render pest virus (Satyavathi et al. 2003) and peste des petits ruminants' virus, respectively (Prasad et al. 2004).

In most of the cases decapitated embryonic axis and cotyledonary nodal tissue were found to be highly responsive and most suitable explants over the other plant tissues for transgenic development (Krishna et al. 2010). Simultaneously, choice of specific promoters were also proved to be crucial in genetic transformation for regulating the activity of foreign genes in host plant tissue and also influence the expression level in spatio-temporal manner (Kummari et al. 2020). The great majority of studies conducted in pigeonpea thus far, have largely relied on the *Cauliflower mosaic virus35S* (*CaMV35S*) constitutive promoter (Ghosh et al. 2014a). Beside this, reports are also available on usage of tissue specific promoters like, flower and leaf specific double enhanced *CaMV35S* (*CaMV35S*DE) promoter and seed specific *phaseolin* and In pigeonpea *Arabidopsis thaliana 2S2 albumin* promoters to drive the tissue specific transgene expression (Sharma et al. 2006; Thu et al. 2007). In spite of prolong practices, the selection mediated in vitro tissue culture based strategies exhibited several bottlenecks like, small fraction of totipotent cells surviving after transformation, antibiotic selection pressure reducing overall regeneration potential of explants and poor rooting responses in tissue culture (Ghosh et al. 2014b).

Under such circumstances, introduction of tissue culture independent in planta transformation strategy in pigeonpea provided a broad avenue in transformation technology for the improvement of this recalcitrant crop (Rao et al. 2008). In this strategy in vitro co-cultivation and selection steps were entirely deviated to generate a huge numeral transformants of pigeonpea. The method was successfully used by Ramu et al. (2012) and Kaur et al. (2016) to express pest tolerant genes in transgenic pigeonpea. Ghosh et al. (2014b) applied grafting strategy in pigeonpea transformation to avoid the rooting problem, which was further successfully used for developing Cry1Ac and Cry2Aa pigeonpea transgenic lines with stable DNA (Ghosh et al. 2017). Further, an alternative tissue culture independent approach called 'plumular meristem transformation' used in pigeonpea with a 72% transformation frequency (Ganguly et al. 2018). This plumular meristem transformation system involved in vitro *A. tumefaciens*-mediated transformation of explants followed by tissue culture independent plant establishment, polymerase chain reaction based screening of T_0 events and antibiotic selection of T_1 transgenic events for their establishment in soil (Ganguly et al. 2018).

6.11.2 Genetic Resources in Pigeonpea for Development of Abiotic Stress Tolerant Transgenic Plant

Pigeonpea demonstrated activation of complex signalling pathways under abiotic stress conditions which induce changes in gene expression profiles of the plant. Few genes are essential for the plants to adjust and acclimatize during the stress conditions. Under temperature and drought stresses pigeonpea exhibits higher expression of some of the key regulatory genes like *CcCYP*, *S-adenosyl methionine synthetase* (*SAMS*), *glutathione S-transferase* (*GST*), *ascorbate peroxidase* (*APX*), *CcHyPRP*, *CcCDR*, and *ABCG* transporters, whereas salt and heavy metal stresses induced *C. cajan metallothionein 1* (*CcMT1*) and *glutathione reductase* (*GR*) (Radadiya et al. 2016; Niu et al. 2021).

Few of these genes were involved in generation of stress tolerant transgenic events in other plant species (Table 6.11). According to reports *CcCYP* gene was found to exhibit higher expression level in pigeonpea plants during drought and salt stresses (Handschumacher et al. 1984; Wang and Heitman 2005). Cyclophilins are ubiquitous proteins that belong to immunophilin chaperon family. These proteins exhibited peptidyl-propyl cis–trans isomerase activity, and catalysed cis–trans interconversion of X-proline peptide bonds (where X is any amino acid residue). Overexpression of *CcCYP* gene in *A. thaliana* transgenic plants was found to be effective for improved level of drought, salt and temperature tolerances in comparison to control treatment (Sekhar et al. 2010). Based on experimental reports by Sekhar et al (2010), T_3 transgenic events showed a survival rate of 95–97% in comparison to control plants with 60% survival rate when they were subjected to drought stress using 300 mM mannitol. These transgenic events exhibited 60–68% increase of biomass after drought stress with respect to control plants. Similarly, in salt stress experiment using 100 mM NaCl, the same transgenic events showed 75–85% survival rate with 119–216% increase in biomass compared to control plants, which showed 40% survival rate. All transgenic events were grown under salt stress showed higher accumulation of Na^+ ion (3.6–3.9 mg/g dry weight) than non-transgenic controls (2.5 mg/g dry weight).

Another report demonstrated the expression of *CcCDR* gene in transgenic *A. thaliana* events, shows high tolerance against the salinity and drought stresses (Tamirisia et al. 2014). The mode of action of this gene is not characterised yet. T_3 homozygous transgenic events constitutively expressing CcCDR showed 80–85% survival rate during drought stress with hiher survival rate. Cold stress of 4 °C showed 80–90% survival rates of T_3 transgenic events than control plants. All these abiotic stress experiments on CcCDR expressing *A. thaliana* transgenic plants revealed 2.5–3 times increase of total biomass, improved chlorophyll content and profuse root growth in comparison to control plants. These transgenic plants demonstrated the abiotic stress tolerance through the increased production of antioxidants, prolines and reducing sugars.

Sunitha et al. (2017) also reported about the tolerance properties of gene *CcCDR* under the control of *CaMV35S* and stress inducible rd29A promoters in transgenic rice against drought, cold and salinity stresses. T_4 homozygous transgenic rice events

Table 6.11 Genetic resources for transgenic abiotic stress tolerance in pigeonpea used

Source plant	Name of the gene	Transgenic plant	Activity of the gene	Transgenic generation analysed	Transgenic traits	References
Cajanus cajan	*CcCYP*	*Arabidopsis thaliana*	Peptidyl-propyl cis–trans isomerase	T_3	Drought, salinity and high temperature stress tolerance	Sekhar et al. (2010)
	CcMT1		Cysteine-rich metal-binding proteins		Metal stress (Cu and Cd) tolerance	Sekhar et al. (2011)
	CcCDR		Abiotic stress regulator		Cold, drought and salinity stress tolerance	Tamirisia et al. (2014)
	CcHyPRP	*Oryza sativa*	Cell wall structural gene	T_4	Drought, salinity and heat stress tolerance	Mellacheruvu et al. (2016)
	CcCDR		Abiotic stress regulator		Cold, drought and salinity stress tolerance	Sunitha et al. (2017)
Pisum sativum	*Psp68*	*Cajanus cajan*	DEAD-box helicase	T_1	Salinity stress tolerance	Neha (2019)
Oryza sativa	*OsRuvB*		DNA helicase		Salinity stress tolerance	Singh et al. (2020)

showed seed germination rate higher by 85–89% under drought (250 mM mannitol), 74–81% under salt (250 mM NaCl) and 85–90% under cold (4 °C) stress conditions compared to control plants. T_4 lines also exhibited survival rate of 80–85% in drought, 85–90% in salt and 90–100% in cold stresses compared to control plants with 40–50% survival rate. Transgenic rice plants also showed 1.6–2.1 fold increase of biomass in transgenic lines along with 1.4–1.6 fold increase in total chlorophyll content compared to control plants. Beside these, CAT activity and SOD activity also increased by 0.5–1.5 fold and 1.4–1.7 fold, respectively which provided effective tolerance against abiotic factors induced oxidative stress.

High concentration of essential and non-essential metal like copper (Cu) and cadmium (Cd) are responsible for toxic effects in cell and inhibition of plant growth. High expression of *CcMT1* gene was reported in pigeonpea during metal stress

conditions (Priyanka et al. 2010a, b). Metallothioneins are low molecular weight (4–14 kDa), cysteine-rich metal-binding proteins which are present in range of organisms. The overexpression of *CcMT1* gene was found to be effective for improved Cu and Cd tolerance in transgenic *A. thaliana* events. T_3 transgenic plants were subjected to both Cu and Cd stresses at two different concentrations (50 and 100 μM) of $CuSO_4$ and $CdSO_4$. Transgenic events demonstrated improved survival rates with increased biomass at Cu and Cd stress conditions in comparison to control plants (Priyanka et al. 2010a, b; Sekhar et al. 2011).

6.11.3 Transgenic Pigeonpea Development for Abiotic Stress Tolerance

Researchers tried to develop transgenic pigeonpea against abiotic stresses (Table 6.12) using tolerance genes identified in well-characterised systems like rice, tobacco and *Arabidopsis*, but the achievements are limited. DNA helicases act as molecular motor in numerous cellular mechanisms in plants and are crucial for almost all metabolic activities at DNA level (Tuteja and Tuteja 2004). *Oryza sativa RuvB* (*OsRuvB*) gene was reported as a DNA helicase which played a key role in tolerance to salt rice (Wang et al. 2011; Saifi et al. 2018)**.** Singh et al. (2020) reported that, incorporation of *OsRuvB* gene in pigeonpea under *CaMV35S* promoter via *Agrobacterium*-mediated transformation method demonstrated improved salt tolerance in T_1 generation. *OsRuvB* transgenic pigeonpea plants showed double survival rate after 15 days of germination under 75 mM NaCl stress compared to wild type plants. T_1 transgenic events showed healthy growth with greener leaves under the mentioned salt stress, compared to the control ones which exhibited poor growth with brown and wrinkled leaves. Also, chlorophyll content in T_1 lines increased by sixfold than control plants after 8 days of 75 mM NaCl stress treatment. Transgenic pigeonpea plants also exhibited less decline in relative water content (average 8.9%) than wild type lines (average 19.9%), increased level of CAT (0.68–4.6 fold) and POX (1.50–6.25 fold) activity compared to control plants after 4 days under salt stress.

Another report was found in pigeonpea, where transgenic expression of *Pisum sativum p68* (*Psp68*) gene showed high level of tolerance against the salinity stress (Neha 2019). The gene is a prototype member of DEAD-box helicase and found to interact with Ca^{2+}—calmodulin regulating diverse signalling pathways during salt stress to increase the tolerance in plants (Wang et al. 2013). Psp68 protein was found to enhance the scavenging capacity of reactive oxygen species in T_1 transgenic pigeonpea lines after 4 days treatment in 75 mM NaCl, through increment of CAT and POX activity by 2.5 and 2.14 fold, respectively, compared to wild type plants. T_1 transgenic events exhibited the 1.5–2.5 fold increase in chlorophyll content after 8 days of 75 mM NaCl stress in comparison control plants. After 8 days of salt stress treatment, relative water content increased by 1.99 fold in transgenic lines, compared

Table 6.12 Genomic resources in pigeonpea

Resource	Type	References
BAC-based resources	88,860 BAC-end sequences (BESs)	Bohra et al. (2011a, b)
	Two BAC libraries comprising 34,560 and 34,560 clones	
DNA based Markers	SSR	Burns et al. (2001), Odeny et al. (2007a, b, 2009), Saxena et al. (2010a), Bohra et al. (2011a, b, 2017), Dutta et al. (2011)
	SNPs	Kumar et al. (2016), Saxena et al. (2012, 2017a, b, c, 2018a, b)
	DArT assay	Yang et al. (2006, 2011)
	Single feature polymorphisms	Saxena et al. (2011)
	ISR	Kudapa et al. (2012)
	Large structural variations (CNV, PAV, InDels)	Kumar et al. (2016), Varshney et al. (2017)
High-density genotyping platforms	Illumina BeadXpress	Roorkiwal et al. (2013)
	GoldenGate	Kassa et al. (2012a, b), Kumawat et al. (2012)
	KASP	Saxena et al. (2012)
	GBS	Saxena et al. (2017a, b, c, 2018b)
	RAD	Arora et al. (2017)
	50 K Axiom *Cajanus* SNP Array	Saxena et al. (2018a)
Transcriptomic resources	ESTs	Priyanka et al. (2010a, b)
	Transcriptome assemblies	Dutta et al. (2011), Dubey et al. (2011a, b), Kudapa et al. (2012)
	Reference genes for expression analysis	Sinha et al. (2015a, b)
	Gene expression atlas	Pazhamala et al. (2017)
	Population specific	Gnanesh et al. (2011a, b), Saxena et al. (2012, 2017a, b, c, 2018b)
Genetic maps	Consensus	Bohra et al. (2012), Arora et al. (2017)
Trait-associated DNA markers	CMS restoration	Bohra et al. (2012); Saxena et al. (2018a, b)
	Fusarium wilt	Singh et al. (2016a, b, 2017), Saxena et al. (2017b)

(continued)

Table 6.12 (continued)

Resource	Type	References
	Sterility mosaic disease	Gnanesh et al. (2011a, b), Singh et al. 2016a, b, 2017b, Saxena et al. (2017a),
	Plant type/growth habit	Kumawat et al. (2012), Mir et al. (2014), Saxena et al. (2017c)
	Seed traits (protein content/size)	Obala et al. (2019); Yadav et al. (2019)
Orgenellar genomic resources	Mitochondrial genome assemblies	Tuteja et al. (2013)
	Mitochondrial DNA markers (SSRs and Indel)	Khera et al. (2015), Sinha et al. (2015c)
	Chloroplast genome assemblies and SSRs	Kaila et al. (2016)
Whole-genome sequencing/resequencing	Reference genome sequence	Singh et al. (2011), Varshney et al. (2012a, b)
	WGRS	Kumar et al. (2016), Singh et al. (2016a, b, 2017b), Varshney et al. (2017)
Modern genetic populations	MAGIC	Pazhamala et al. (2015)
	NAM	
Genetic purity testing kits	SSR assay	Saxena et al. (2010a, b), Bohra et al. (2011a, b, 2017)

to wild type plants. At the same time, total soluble sugar level also increased by 2.2 fold, which maintained cell homeostasis by acting as osmolytes (Neha 2019).

6.11.4 Future Prospects

Several stress factors are major concern that destabilizes the pigeonpea productivity in agricultural ecosystem. The recent advancement of recombinant DNA technology has proven to be extremely beneficial for crop improvement by including functional genomics approaches, laying the groundwork for an advanced agriculture system with built-in resilience to achieve maximum output. Several tolerance genes were identified with potential for the development transgenic abiotic stress tolerance through overexpression or inducible expression based strategies. Genome editing tools can be introduced in pigeonpea for modifying the activities of key regulatory genes involved in stress tolerance. The application of plumular meristem transformation protocol was proved to be effective for overcoming the recalcitrancy of this

particular legume for transgenic development. Choice of tissue specific and inducible promoters will be a major concern for transgenic development.

6.12 Brief Accounts on Role of Bioinformatics as a Tool

6.12.1 Genomic Resources

The creation of large-scale genetic resources in pigeonpea has had a catalytic influence on its improvement over the previous ten years (Table 6.12). More than 3,000 SSR markers in pigeonpea are already available (Varshney 2016). Other types of markers, such as DArT markers in pigeonpea are also available (Yang et al. 2011; Thudi et al. 2011). Similarly, varied technologies involved in recognizing millions of SNP and InDel markers (Varshney et al. 2012b; Deokar et al. 2014; Das et al. 2015). In addition, cost-effective marker genotyping assays have been developed, including KASP assays (Saxena et al. 2012; Hiremath et al. 2012), GoldenGate assays, and VeraCode tests (Roorkiwal et al. 2013). The Affymetrix SNP platform was recently used to create 60 K SNPchips for pigeonpea (Saxena et al. 2016a, b).

6.12.2 Comprehensive Transcriptomic Resources

Pigeonpea genetic molecular markers were developed for genetic study and breeding purposes. The initial set of transcriptome resources, consisting of 9468 high-quality ESTs, was created using Sanger sequencing of cDNA libraries to identify potential genes for fusarium wilt (FW: 19 genes) and sterility mosaic disease (SMD: 20 genes), as well as a set of 3583 SSRs. A variety of transcriptomic resources have benefited from the recent movement from traditional gene expression methodologies to digital platforms. Using the Illumina and FLX/454 technologies, several transcriptome assemblies comprising 21,434 transcript assembly contigs (TACs), 48, 726 TACs and 43, 324 TACs (Kudapa et al. 2012; Dubey et al. 2011a, b; Dutta et al. 2011) have been described in pigeonpea. Recent pigeonpea gene expression research has shed light on the plant stress response and provided a collection of stable reference genes to assist expression investigations under drought, heat, and salinity stress conditions (Sinha et al. 2015a, b). To attain a consistent rise in pigeonpea productivity, existing breeding efficiency must be improved. Modernization of breeding programs will rely heavily on innovative breeding plans supported by relevant genomic technology.

6.13 Brief Account on Social, Political and Regulatory Issues

6.13.1 Patent and Intellectual Property Rights (IPR) Issues

Intellectual property rights are anticipated to play an important task in providing economic benefits for the intellectual and financial investments that make research and development possible in agriculture, given that many nations have implemented or are in the process of passing IPR legislation. Grain and legume crop fertility and reproduction have long been associated with spiritual significance throughout Africa, Asia, and parts of the Americas (Nuffiel Council on Bioethics 1999). Plant variety protection through IPR has an impact on seed prices, farmer rights (as seed consumers, breeders, and biological resource conservators) and, as a result, global food security (Murdock et al. 2008). Providing different varieties of plant by individual, public, and corporate sources, as well as the research partnership sector, are encouraged to participate in varietal development research by providing them with the incentive of exclusive control for specified time, allowing them to regain their research investment (Prasanna et al. 2019).

Because of heterosis (hybrid vigour), most pigeonpea growers prefer plant hybrids that are more uniform and vigorous than conventional kinds and these advantages are lost when second generation seed is employed. Despite the fact that pigeonpea is important grain legume crops across the globe, no pigeonpea transgenics have yet to be commercialised. As a result, intellectual property rights must be adjusted to the real extent of new genetically modified (GM) discoveries in order to avoid impeding ongoing research, innovation and development of this vital pulse legume (Murdock et al. 2008). Researchers and farmers are excluded from the PVP system which resulted that PVP is less effective as an IPR than patents. This claim, however, is irrelevant in the context of hybrids (Bhutani 2011). As a result, it's important to keep a close eye on upstream PVP ownership (particularly with regard to hybrids), the downstream seed market (Prasanna et al. 2019) and technology/trait licensing policies.

6.13.2 Farmers Right

Many farming communities, particularly small farmers in developing nations, continue to sustain a dynamic process of crop conservation and development despite agricultural modernisation. Farmers play a part in this evolution process, whether consciously or unconsciously, by cultivating crops in local agro-ecosystems and selecting and exchanging seeds. Natural selection by means of selection force at field level via various mechanisms, conscious farmers select using many different plant characteristics to identify and select their crop varieties, which aids gene flow

and introgression into the growing population (Smolders 2006). During 2001 IPR restrictions were introduced in India to protect the interests of breeders and farmers.

6.13.3 Participatory Plant Breeding (PPB)

Participatory Plant Breeding has the potential to increase crop quality in farming communities. The goal is to create regionally tailored crop improvement methods in order to increase crop genetic diversity conservation and usage. Farmers' varietal preferences were identified. This information is critical for developing effective strategies for durable production system in pigeonpea. Farmers' need based features in pigeonpea varieties will also included while selecting varieties or advanced breeding materials to be introduced into participatory varietal selection programmes in order to improve the crop's production in Benin (Ayenan et al. 2017). The PPB procedures, which promote circumstances for farmer-researcher cooperation in plant breeding for agricultural crop improvement, highlight the significant benefit of information interchange and shared learning between researchers and farmers.

6.14 Future Perspectives

6.14.1 Potential for Expansion of Productivity

To achieve a stable increase in pigeonpea productivity, current breeding efficiency must be improved. The modernization of breeding programmes will rely heavily on innovative breeding designs supported by appropriate genomic technologies. Reduced crop breeding cycle length combined with improved selection intensity holds the key to improving genetic gains accrued from breeding programmes in a crop like pigeonpea that shows significant maturity generation with photoperiod sensitivity. Though genomic selection models and speed breeding protocols will play an important role, strengthening germplasm collection programmes and seed delivery to boost productivity. In terms of crop improvement, the diverse genetic resources utilization in breeding programmes and genomic diversity perceptive at gene pools are critical (Bohra et al. 2020). Conventional methods rely on deployment of existing natural genetic diversity to carry out breeding programmes, but the results are frequently skewed due to genotype–environment interactions. Even multi location data cannot completely eliminate this bias, which may result in some selection inefficiencies and high throughput phenotyping technologies are able to address these issues by generating precise data from large scale measurements using latest crop phenomics strategies.

Application of DNA marker technologies, contributed to the significant shift in the procedures used to estimate molecular diversity using genotypic data. Molecular

marker data, on the other hand, has been deemed unsuitable for providing information about genetic diversity of key adaptive traits (Jackson et al. 2011). Gene-based functional markers and more recently, WGRS/skim sequencing of diverse accessions have been used to estimate genetic diversity in pigeonpea. Prior to the whole-genome sequencing and re-sequencing of pigeonpea, SNP-based analysis of a variety of germplasm, including wild species, revealed a severe "domestication bottleneck" in the plant (Kassa et al. 2012a, b). The use of high-density SNP arrays and re-sequencing data in recent research has revealed a trend in genetic diversity and breeding targets in legumes (Bohra and Singh 2015). To improve pigeonpea yield through climate adaptation, it is essential to reintroduce the diversity that was gone during process of domestication and selection (Kumar et al. 2016). In addition, high-density genotyping/sequencing information from a variety of germplasm panels can be combined with phenotypic data to uncover new breeding trait-associated alleles. Breeding techniques such as speed breeding, genomic selection, and MABC helps in speed up the identification and introgression of these valuable alleles into adapted germplasm (Li et al. 2018). In view of the above, pigeonpea improvement need to address the farmers demand with advanced breeding methodologies by including molecular & genome based technologies (Saxena et al. 2016a, b).

6.14.2 Potential for Expansion into Nontraditional Areas

The majority of previously released pigeonpea varieties were developed through landrace selection. To meet crop improvement challenges, efforts were made to widen the genetic base by collecting and conserving germplasm from around the world before it was lost forever, resulting in the creation of large collections at national and international gene banks (Upadhyaya et al. 2013). However, to support the food security and economic health of agriculture-based countries, the cultivation of climate-smart crop genotypes, as well as the implementation of effective measures to reduce global warming, is now required. Plant breeders must now work faster to identify/develop germplasm lines/varieties that can withstand or benefit from climatic anomalies such as temperature extremes, moisture stress, disrupted rainfall patterns and increased CO_2 levels. While dealing with the effects of climate change, disease and insect pest resistance is also a priority to ensure yield stability (Bahl 2015). This allows us to expand production into nontraditional areas. For greater precision and efficiency in breeding programmes, integrated breeding involving multidisciplinary (genetics, physiology, and biotechnology) approaches is required. The focus of screening and selection should be on reproductive traits. While, drought and heat stress issues are addressed, physiological parameters such as root traits, transpiration efficiency, dehydration tolerance, membrane stability index, and pollen viability should be given due attention in conjunction with yield. Cold tolerance should be determined by survival, anthesis, pollen dehiscence and pod and seed setting at low temperatures. For salinity and acidity, root exclusion and limited shoot translocation of toxic materials along with yield should be considered (Choudhary et al. 2018).

Apart from that, pigeonpea plants are divided into two types: Determinate (DT) and Indeterminate (IDT) (Mir et al. 2013), when short-statured DT types reach flowering, they stop growing, whereas vigorous IDT types keep growing even after flowering. Despite the fact that pigeonpea growers prefer IDT, continuous flowering followed by nonsynchronous harvesting draws attention to DT type breeding. Because the DT type has a higher initial vigour, tolerance to drought and waterlogging, and eases of mechanical harvesting than the IDT type, it is necessary to focus on the development of such a variety to meet the needs of marginal farmers.

In present scenario, not only food security but nutritional security is major concerned factors in the development of new genotypes. Productivity efficiency is determined by the total nutrient content of the seed, which must meet the population's needs with minimal waste. Pigeonpea requires more attention towards the improvement of amino acid profiles, particularly the level of sulfur-containing amino acids, and to eliminate anti-nutritional factors. Salinity is becoming a problem in rice and wheat growing areas all over the world. Diversification of cropping systems is very important, especially with legumes, as a recommendation. Short-duration pigeon pea varieties found beneficial in a rotational cropping system (Saxen 2008) and breeding for extra early genotypes must be accelerated. Other than this, traditional genotypes require a short day for flowering; crop adoption is limited to 30° North and South latitude. Genotypes that are less photoperiod sensitive and mature in 90–120 days have shown adaptation to latitudes ranging from the equator (Kenya) to 46° N (Prosser USA) and 45° S in New Zealand (Saxen 2008), which may aid in pigeonpea productive area expansion. Based on the available literature, it appears more efforts required to improve pigeonpea abiotic stress tolerance. The current challenges in pigeonpea cultivation are to narrow the gap between potential and realized yield and reduced yield differences in major growing regions, where the abiotic stresses mentioned in Sect. 6.2 are prevalent. The development of resistant/tolerant cultivars with consistent performance across environments is required for holistic supervision of abiotic stresses. More efforts are needed to develop high-yielding stable cultivars because environment and GE interaction contributes for nearly 95% of the overall variation in pigeonpea (Choudhary et al. 2011). In the days ahead, combining phenotype-based advanced genomic tools with more efficient screening methods will be particularly important in making pigeonpea cultivation a promising, profitable and viable option for pulse-growing farmers around the world.

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Chapter 7
Genetic and Genomic Research for Abiotic Stresses in Faba Bean

Fouad Maalouf, Lynn Abou Khater, Zayed Babiker, and Amel Mohamed

Abstract Faba bean is an important legume grown in diverse cropping systems in many regions. The crop suffers from diverse abiotic stresses which reduce the yield and limit its expansion to new niches. The major abiotic stresses are extreme temperature, drought, waterlogging, acidic soils and salinity. Breeding efforts for tolerance to abiotic stresses resulted in the development of cultivars for cold, heat, drought, and acidic soils for diverse environments. These were the results of traits deployment in the field which require long time. The application of modern speed method is considered one of pioneer innovation to ensure significant increase of the genetic gains and consequently the efficiency of the breeding programs. The genome wide association studies will be useful for the identification of efficient markers that can be used in the breeding program. This will open the scope to conduct precise screening for abiotic stress related traits in larger population in early generation allowing the shortening of breeding cycle and the increase of the selection intensity which will be expressed in higher attainable genetic gains in faba bean.

Keywords Faba bean · Heat · Drought · Salinity · Soil acidity · Genomic research · Proteomic

7.1 Introduction

Faba bean (*Vicia faba* L.) is one of the widely grown food legumes with average world production of 4.9 million tons gown in 2.5 million ha in 2017–2019. The global area declined from 5.4 to 2.1 million ha between 1964 and 1992 mainly in developing countries and since then the area increased to roughly 2.5 million ha

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(Fig. 7.1). The global area recuperated is therefore around 400,000 ha over last 28 years which represent the expansion of faba bean is East Africa, Oceania, Europe and North America. The global average yield stands around 2 tons/ha which is the highest among cool season legumes in the world (FAO 2021). The faba bean crop is mostly cultivated for its local consumption in South and East Asia, North and East Africa, and South America. In Mediterranean countries faba bean is mainly used for vegetable purposes and for dry seeds. The productivity per area has tended to increase from early 1980 due to the adoption of improved cultivars with resistance to biotic and abiotic stresses (Link et al. 2010; Sillero et al. 2010; Singh et al. 2012; Maalouf et al. 2019). The total production of faba bean grain in 2019 was approximately 45% greater than in 1992 (FAO 2021). The faba bean are highly nutritious because they have a high protein content (from 18 to 35% in dry seeds), and are a good source of many nutrients, such as K, Ca, Mg, Fe, and Zn (Longobardi et al. 2015; Neme et al. 2015).

In other hand, faba bean plays an important agronomic role through its ability to fix nitrogen by symbiosis with Rhizobium bacteria, even in the presence of high amounts of available N in the soil. In fact, several studies have demonstrated savings up to 100–200 kg/ha^{-1} of N fertilizer required to maximize the yield of crops grown after faba bean (Jensen et al. 2010). Therefore, the cultivation of faba bean as a rotation crop has great benefits. It can enhance phosphorus availability, microbial community in the rhizosphere and soil nitrogen for subsequent cereal crops (Yigezu et al. 2019).

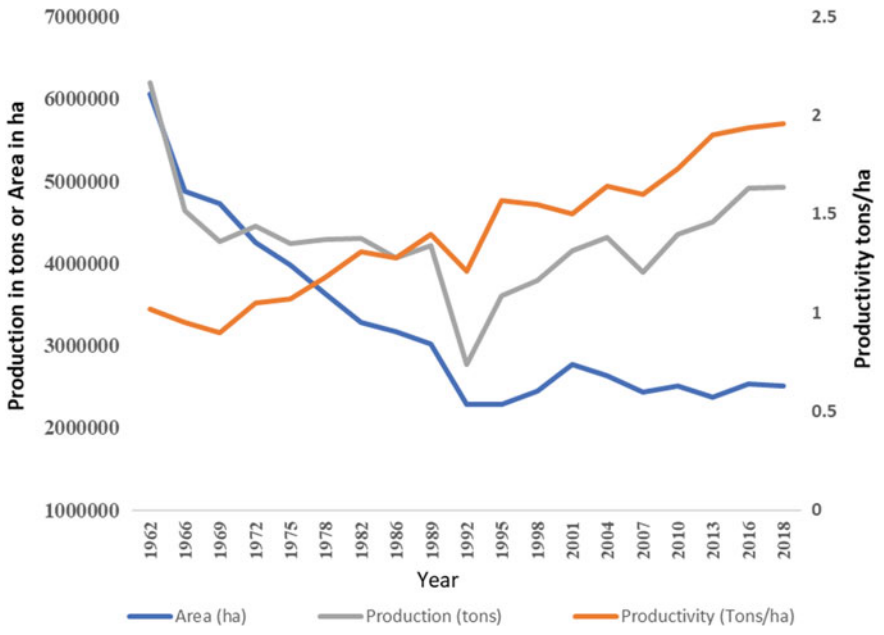

Fig. 7.1 Trends of global area, production, and productivity (FAOSTAT 2020)

Despite the economic and agronomic importance of faba bean and strong cultural attachment to Mediterranean basin, Middle Eastern, Nile Valley and East African populations, many of these countries have become net importer. Currently, there is significant shortage in production of faba bean in North Africa. The North African countries imported more than 0.34 million tons in 2012–2013 (Nedumaran et al. 2015). Due to the high population growth rates, Africa and the Middle East are projected to have the strongest growth in food demand and trade over the coming decade. Its shortage is associated to diverse biotic and abiotic stresses. In the following paragraph we will focus on the major abiotic stresses that affect the production of faba bean.

The climate change scenario intensified with more frequent high temperatures, rainfall pattern changes, with the continued temperature extremes specially in North and East Africa where faba bean is the major cool season food legume. Thus, the major abiotic stresses that affect the faba bean production are heat, drought, waterlogging and frost in many production regions around the world. The rainfall irregularity is the major cause of faba bean yield reduction (Siddique et al. 2001). Temperature above 35° during flowering stage affects drastically the production of faba bean (Patrick and Stoddard 2010; Bishop et al. 2016). In addition, frost damage affects the yield performance in Northern countries of Europe and America (Landry et al. 2015, 2016). For East Africa, the waterlogging and acidic soils are the limiting factors specially in high Ethiopian lands (Keneni et al. 2010). The genetic improvement of abiotic stress tolerance in faba bean is therefore one of the major objectives for different target environments. For North Africa, the major focus is drought tolerance while for East Africa is heat tolerance and tolerance to acidic soils (Maalouf et al. 2019). In northern part of Europe drought and freezing tolerance are the key traits (Stoddard et al. 2006). The breeding techniques are mainly fields and controlled based experiments under normal conditions and under growth room, respectively. This chapter therefore cover the major abiotic stresses, major achievement to overcome these stresses, breeding methods and recent advances on proteomics and genomics research.

7.2 Major Abiotic Stresses

Heat, drought, waterlogging and cold are the major abiotic stresses affecting the faba bean productivity in North, East Africa and West Asian countries and are briefly described as follows.

7.2.1 Cold Stress

In cool-temperate regions such as in Northern Europe, faba bean is mainly grown during spring period despite the higher yield potential when grown in winter. This is because of the low tolerance of cold winter accessions (Arbaoui et al. 2008).

Shifting the faba bean cultivation to winter period allows the crop to rely in available moisture in the soil by maturing before season in summer starts (Link et al. 2010). However, winter sowing can only be achieved by using highly cold tolerant cultivars in the target environment. This technology allows early planting and lead the crop to escape terminal drought (Bond et al. 1994; Link et al. 2010). Cold-related stress could be defined in terms of either chilling (between 0 and −12 °C) or freezing (below 0 °C) without snow cover (Toker et al. 2007). ICARDA has identified several germplasms that are tolerant to cold at during cool season at Aleppo Syria from 2009 to 2012.

7.2.2 Heat Stress

Heat stress is very harmful at the reproductive stage of faba bean plants. Its yield is reduced when temperatures raised to 30 °C (Lavania et al. 2015). Heat stress during flowering and pod sets can cause reductions in key yield parameters of faba bean (Bishop et al. 2016). In fact, high sensitivity of pollen to heat stress in faba bean is one the key parameter which is shown also in many other legumes and other plant species (Prasad et al. 2006). The optimum temperature for flowering period of faba bean is 22–23 °C while lower temperature than the optimal during early plant growth can delay the flowering time (Patrick and Stoddard 2010).

7.2.3 Drought Stress

In rainfed agriculture, faba bean depend on rainfall and soil moisture. Faba bean respond well to irrigation (Maalouf et al. 2013) but it is more sensitive to drought than other grain legumes such as lentils (*Lensculinaris*), grasspea (*Latyrus sativus*) and chickpea (*Cicerarieterium*) (Daryanto et al. 2015). Drought stress faba bean reduce yield and biomass and can affect the efficient and sustainable development of agriculture. In drought-prone regions of North and East Africa, a shortage of water, especially during the flowering period, can cause significant yield reduction (Belachew et al. 2019). Escape from terminal drought is one of the adopted strategies in many semi-arid environments (Loss and Siddique 1997) through the development of early flowering lines. The genetic variation in root and shoot responses to water deficit irrigation has been observed at early vegetation (Belachew et al. 2018) and at the flowering time (Khan et al. 2007; Khazaei et al. 2013; Maalouf et al. 2015). At seedling stage, faba bean plants exhibiting deeper and wider root system to avoid drought by water is scare (Belachew et al. 2018).

7.2.4 *Acidic Soils*

Soil acidity is the major limiting factors of the production of legume crops in general and faba bean in high Ethiopian lands (Agegnehu and Fessehaie 2006; Mesfin et al. 2019). The acidic soils occupy about 40% of the total arable land area in the world (Kisinyo et al. 2014). Soil acidity affects the sustainable crop production in the Ethiopian highlands, where the rainfall intensity is high and crop cultivation has occurred with reduced manure application, and removal of crop residue (Melese et al. 2015; Fekadu et al. 2017). This led to a significant reduction of the available nutrients, such as P, Ca and Mg (Dodd and Mallarino 2005), or increase toxicity of Al, Fe and Mn.

7.2.5 *Soil Salinity*

Salinity is one of the major abiotic stress factors threatening agriculture sustainability in general and limiting the expansion of legume crops in major affected areas. The United Nations Environment Program estimates that approximately 20% (Yamaguchi and Blumwald 2005) of agricultural land and 50% of cropland in the world is salt-stressed (Flowers and Yeo 1995). In saline prone environments, faba bean as other pulse crops tend to be more affected than the cereals (see the classification of Ayers and Westcot 1985; Francois and Maas 1994; Maas and Hoffman 1977; Maas and Grattan 1999; Katerji et al. 2001). The reason for this high sensitivity in faba bean can be explained by several factors. The first reason is that faba bean has an undetermined growth habit and longer reproductive stage than the cereals (Katerji et al. 2005). In addition, Rhizobia in the soil is affected by high sodic chloride which affect the symbiotic nitrogen fixation of faba bean (Pessarakli et al. 1989; Singleton et al. 1982; Alexander 1984) and the formation of efficient nodulation (Rai et al. 1985; Craig et al. 1991). Therefore under saline conditions, the number and weight of nodules per plant are also reduced (Elsheikh and Wood 1990; Delgado et al. 1994; Saadallah et al. 2001; Cordovilla et al. 1995a, b; Katerji et al. 2011; Bhattacharya et al. 2019).

7.3 Breeding Efforts

The major breeding objectives in faba bean is to increase yield and yield stability under diverse environments. In addition to improve yield and yield stability, improving poor mechanization, susceptibility to biotic and abiotic stresses and presence of non-nutritional factors are other traditional constraints in faba bean cultivation (Torres et al. 2012). Faba bean is susceptible to many biotic and abiotic stresses, which reduce yield and affect yield stability. The abiotic stresses such as drought

and heat are the major constraints of faba bean production (Amede and Schubert 2003; Abdelmula 2007; Maalouf et al. 2019). Screening for major abiotic stresses has resulted in the identification of sources for tolerance to heat, frost drought stresses and tolerance to acidic soils.

7.3.1 Cold Tolerance

Improving cold tolerance of faba bean is essential to improve the productivity in cool temperate environments (Toker et al. 2007), since cold tolerance is considered the main component for winter growing regions (Bond et al. 1994; Arbaoui and Link 2008). Non-destructive methods were proposed to screen faba bean for cold tolerance such as visual scoring of appeared injuries in leaves (Herzog 1987b, 1989; Badaruddin and Meyer 2001), measuring re-growth or leaf conductivity (Herzog 1987b), and evaluating chlorophyll fluorescence (Herzog and Olszewski 1998). Although visual scoring may not be a perfect estimation (Herzog 1987b), it is useful to screen large number of accessions under field conditions. Breeding for frost resistance and winter hardiness in northern Europe (Arbaoui et al. 2008; Link et al. 2010) has resulted in the development of cultivars and improved lines were identified to be tolerant to winter hardiness in Germany, Bulgaria, and other areas with a similar winter climate (Landry et al. 2015; Ali et al. 2016).

7.3.2 Heat Tolerance

The first report on the possibility of identification of heat tolerant faba bean was presented by Abdelmula and Abuanja (2007) who confirmed the tolerance of the Sudanese faba bean accession C.52/1/1/1 to heat. In addition, heat stress at temperature above 32 °C can lead to the reduction of the percentage of germinated seeds, increase of abnormal seedlings, degeneration of nodules affecting the nitrogen fixation efficiency and reduction of plant biomass. Heat stress during the reproductive phase affects pollen viability, fecundation, number of formed pods and seeds and seed size.

Genotypic variation for heat tolerance has been identified in faba bean (Maalouf et al. 2019). Wide range of pollen germination was observed under high temperature (Table 7.1). Heat stress caused significant reduction in pollen germination and yield components of most studied accessions. Selected accessions with high pollen viability in various seasons are presented in Table 7.1. These accessions were also found with high number of pods and seeds under heat prone conditions.

In addition five released cultivars for heat tolerance were evaluated under continuous heat with maximum daytime temperature above 35° in Aljazeera Scheme in two consecutive year showed that Hudeiba 93 and Ed-Damer cultivars are the most tolerant (Table 7.2). These finding open the scope to out-scale faba bean in Aljazeera

Table 7.1 Pollen viability (%) of selected accessions evaluated under summer season in 2015, 2016 and 2017 at Terbol station, Lebanon

	2015	2016	2017
Average	9.5	16.3	10.6
Mean	0.0–50	0.0–72	0.0–56
	Pollen viability of selected accessions		
FB1197	**28**	**26**	**30**
FB1482	15	**24**	17
FB2047	15	**35**	**45**
FB2077	15	**26**	**34**
IG11908	**50**	**20**	15
IG13771	15	**22**	**20**
VF683	**20**	15	**30**
VF729	**20**	15	**23**

Table 7.2 Evaluated faba bean cultivars under continuous heat stress in Aljazeera scheme

	2017/18		2018/19	
	Grain yield kg/ha	Hundred seed weight	Grain yield kg/ha	Hundred seed weight
Shendi	1816	54	1299	44
Hudeiba 93	**2034**	48	1077	42
Basabeer	**2294**	53	1122	43
Ed-Damer	**2307**	51	**1323**	45
Merowe	2104	48	1244	46
Mean	2111	51	1150	40
LSD at $p < 0.05$	812	4	282	4.1

scheme. The severe heat during 2018/2019 at Jazeera scheme induced yield reduction of all cultivars, also the seed size of all accessions was reduced under heat stress. However, Ed-Damer cultivar was the most tolerant to heat among all tested cultivars in Sudan.

7.3.3 Drought Tolerance

Field- and controlled-condition experiments are conducted to screen faba bean tolerance to drought (Stoddard et al. 2006) with consideration of physiological issues such as ^{13}C discrimination. Khan et al. (2007) concluded experimental evidence pointing to stomatal conductance, leaf temperature and ^{13}C discrimination as promising tools

for breeding drought tolerant faba bean and proposed ^{13}C discrimination to assess transpiration efficiency in faba bean. Another study conducted by Maalouf et al. (2015) proposed the use of spectral indices structure-insensitive pigment index and normalized preoptimization index for selection under drought-prone environments because they were positively correlated with grain yield under dry conditions.

7.3.4 Tolerance to Acidic Soils

Breeding faba bean for adaptation to acidic soils is the major objective of the breeding program for high Ethiopian lands (Gemechu et al. 2016) as the soil management is very costly and has low cost benefit to the farmers (Mussa et al. 2008). Breeding success for acidic soils has been reported by Temesgen et al. (2015) and for waterlogging (Mussa et al. 2008) in faba bean by identifying different materials introduced from ICARDA and collected from areas with high waterlogging. Wide range of variability for tolerance to acidic soils were detected in faba bean. This observation indicates the possibility to screen faba bean genotypes for soil acidity stress tolerance with good probability to identify tolerant accessions to acidic soils. The development of the variety 'Walki' for waterlogged and vertosols areas was possible due to crossing ICARDA lines with local cultivars; this variety is gaining popularity in the central highlands of Ethiopia. Vertosol has high potential to improve crop productivity and increase production in Ethiopia. However, its potential is highly limited due to waterlogging. Reports of USAID project showed that scale-up of improved technologies such as use of waterlogging tolerant varieties accompanied by improved soil drainage using broad-bed and furrows re-introduced faba bean production that was abandoned in most heavily waterlogged Vertosol areas. This is an impressive contribution in the effort of improving productivity and production on the Vertosols, covering about 8 million hectares of the highlands of Ethiopia (Bishaw in personal communication).

7.3.5 Tolerance to Salinity

High salinity causes both hyper osmotic and ionic stress, which results in alteration in plant metabolism including reduced water potentials, ionic imbalances and specific ion toxicity (Tester and Devenport 2003). The salt tolerant faba bean plant (Abdel-Wahab and Zahran 1981; Cordovilla et al. 1995a, b) might be related to the compartmentation mechanisms achieved by plant protoplasm to cope with higher salt concentration (Bulut and Akinci 2010). A salt-water flooding method (Tuyen et al. 2010) was used to evaluate the salt tolerance of the 50 faba bean accessions using 200 mM NaCl salt concentration at ICARDA. Among them 10 accessions were further re-evaluated by applying three salt concentrations (0, 150 and 200 mM NaCl) with three replications. By increasing salinity level, the biological yield g/plant was reduced (Fig. 7.2) Only two accessions (Sel.TH/4002/09, Sel.TH/4199/09) confirmed their

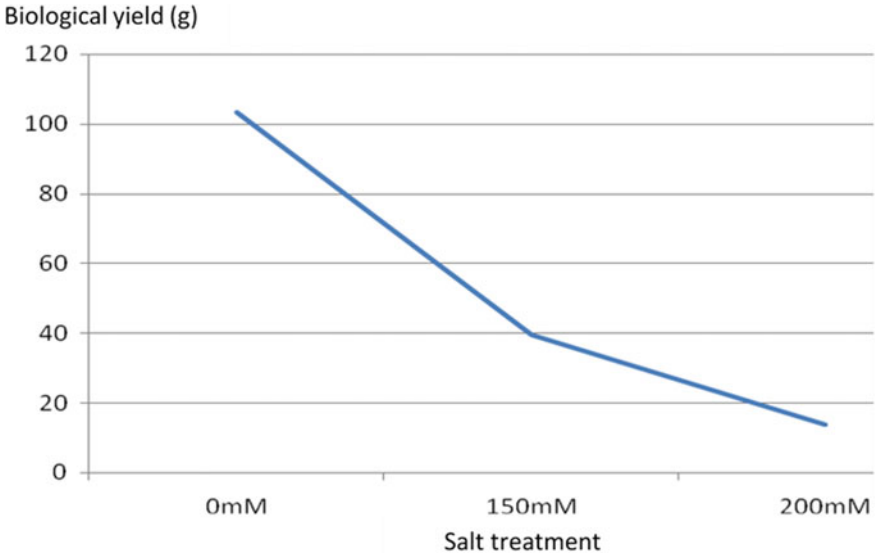

Fig. 7.2 Effect of NaCl concentration on the biomass in g of faba bean accessions

resistance to salinity at 150 mM (Fig. 7.3). These two lines were essential to develop faba bean cultivars moderately tolerant to salinity and may open scope to introduce faba bean in saline prone conditions such as new reclaimed lands in Egypt.

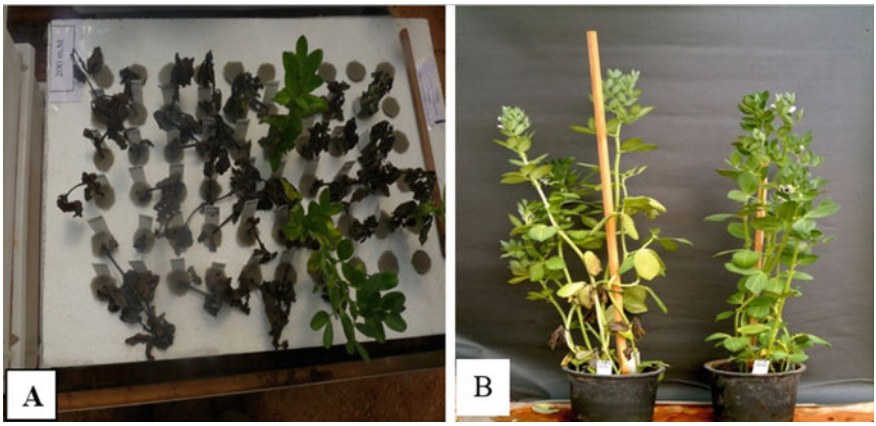

Fig. 7.3 Screening for salinity tolerance. **a** Screening under a salt-water flooding method at 200 mM (Tuyen et al. 2010); **b** testing susceptible check (left hand) and the resistant line (right hand)

7.4 Genetic Resources and Diversity

Vicia faba L. ($2n = 12$) belonging to the subgenus *Vicia* has wide range of variability within the domesticated gene pool, with the major center of diversity located around Mediterranean basin and other centres of diversity in Ethiopia, and Central and Eastern Asia (Duc et al. 2010). This crop is very diverse and was divided into four botanical groups (Cubero 1974), considered earlier by Muratova (1931) as four subspecies which are: "*Vicia faba major*" (large-seeded or "broad" bean above 100 g for hundred seeds), "*Vicia faba equina*" (mid-sized or "horse" bean, 60–80 g), and "*V. f. minor*" (rounded seeds with size with varying from 20 to 50 g/100 seeds) and "*V. f. paucijuga*" (very small, rounded seeds, 20 g/hundred seeds)" types (Maalouf et al. 2013). The domestication of faba bean occurred probably during the late 10th millennium before Christ (BC) at Tell El-Kerkh, north-west Syria (Tanno and Willcox 2006). 14,000-year-old specimens of wild relatives were found in the Mount Carmel region (Caracuta et al. 2016). The medium-sized type was found in the Iberian Peninsula as well as Central Europe 5,000 before present (BP). The larger types were found around 1,500 BP (Ladizinsky 1998). ICARDA conserve the largest collection of faba bean globally with total of 10,320 accessions. ICARDA holds a diverse collections from 71 countries (Redden et al. 2018).

The first report on faba bean genetic variability were published by Abdalla et al. (1976) based on morphological traits and natural variation. The first studies on genetic diversity were based on morphological characteristics and isozyme markers (Jaaska 1997; Polignano et al. 1999). The application of DNA-based markers since the mid-1990s offered great opportunity to assess the genetic diversity in faba bean germplasm (Torres et al. 1993; Zeid et al. 2003; Göl et al. 2017). For example, amplified fragment length polymorphism (AFLP) genotyped data could separate (1) the Asian accessions as distinct as a group from those of European and North African origin (Zeid et al. 2003) and (2) the Chinese germplasm from the germplasm collected outside of China and the winter types from the spring types (Zong et al. 2009). In addition, single nucleotide polymorphism (SNP) markers were used to study genetic diversity within and between faba bean populations and could lead to differentiate Australian accessions based on geographical origin (Kaur et al. 2014).

In addition to the diverse collection derived from generation challenge program (GCP) constructed at ICARDA, the focused identification of germplasm strategy (FIGS) was used as one of approach to identify sets of faba bean that can be screened for the different traits. Environmental parameters describing plant germplasm collection sites are used as selection criteria to improve the probability of uncovering useful variation. FIGS set developed by ICARDA was used to evaluate the diversity of faba bean for traits related to drought adaptation (Khazaei et al. 2013). ICARDA has also developed FIGs sets for heat and cold in faba bean for further use and evaluation under prone conditions.

7.5 Traditional Breeding Methods

Faba bean is a partial allogamous and entomophilous species and can play a critical role in sustainable agriculture and in conserving wild pollinators in natural ecosystems (Maalouf et al. 2019). The outcrossing rates, varying from 4 to 84% (Suso et al. 1999), potentially determines the amount of realized heterosis, which increase yield and yield stability and resistance to abiotic stresses (Gasim and Link 2007). Faba bean breeders are looking forward to determining which breeding strategy is more effective in order to achieve higher genetic gains and consequently can lead to high yielding varieties. Studies on comparison of faba bean breeding strategies have not been widely undertaken in recent years, as the recurrent selection method is barely applied on faba bean populations, whereas pedigree selection and synthetic cultivars are regarded as a major breeding method for this partial allogamous crop (Maalouf et al. 2002; Ibrahim 2015).

The elevated level of cross-fertilization in faba bean (>0.5) is essential for developing synthetic varieties and/or improved open population (Metz et al. 1994). The suitable option is to develop synthetic varieties using autofertile lines to ensure minimum yield in the absence of insect pollinators and to take advantage of their presence by exploring heterosis to increase yield and yield stability (Cubero and Moreno 1984). Both recurrent and synthetic breeding method may lead to exploit heterosis in faba bean cultivars and then enhance yield and yield stability (Link et al. 1994b, 1996; Abdelmula et al. 1999; Arbaoui and Link 2008; Gharzeddin et al. 2019) as well as increase the resistance or tolerance to major abiotic (Gasim and Link 2007; Terzopoulos et al. 2008) and biotic stresses (Maalouf et al. 2008).

Recurrent selection has been used to improve cross-pollinated (Viana 2007) or partial cross-pollinated crops (Odilon et al. 2017) like faba bean. The first report on utilizing recurrent selection method in faba bean was described by Rowland (1987), who revealed that a recurrent selection program consists of growing superior lines in open pollinated random mating nurseries (RMN). From these experiments, well podded plants were selected and; their offspring evaluated for yield (Rowland 1987). The selected lines showed a positive genetic gain of 1.8% per year. It might be possible that most single plants selected in open pollinated conditions were hybrid plants as hybrid faba bean plants are more autofertile than inbred plants (Drayner 1959) and therefore they should produce more pods/plant. This is the main selection criteria used currently at ICARDA breeding program and it is like the method described by Rowland et al. (1986). Gharzeddin et al. (2019) confirmed that higher yields in lines selected by recurrent selection than in lines developed by pedigree method were observed, as the response to selection in recurrent lines was higher than those obtained by pedigree method.

Synthetics can achieve higher yield performance than other open-pollinated varieties as recurrent varieties have received little systematic breeding for yield (Gharzeddin et al. 2019). Classical breeding studies require a longer time to select individual clones than the development of synthetic varieties (Flajoulot et al. 2005). Synthetics usually yields more than lines developed by the recurrent selection method

(Gharzeddin et al. 2019) as heterosis and heterogeneity can be better exploited in faba bean synthetic varieties (Poulsen 1981; Link et al. 1994a, b; Stelling et al. 1994) than in recurrent cultivars.

The pedigree methods are also used in faba bean to develop pure lines with high level of auto fertility. Several authors have proposed in their breeding programs to transform the mating system of faba beans towards autogamy (Adcock and Lawes 1976; Kambal et al. 1976; Bozzini and Chiaretti 1999) to develop inbred lines for organic farms (Ghaouti et al. 2008). However due the strong inbreeding depression (Drayner 1959) through autogamy, it is difficult to achieve high yield by using pedigree breeding method (Lawes et al. 1983). Evaluation of the pedigree method, single seed descend, and mass selection have been conducted by different researchers (Nassib et al. 1978; Hawtin 1982; Ahmed et al. 2008). These methods, which are common methods for self-pollinated crops, revealed that the pedigree method was the most appropriate for faba bean (Ahmed et al. 2008). Selection for a high self-fertility degree might represent an important advantage for simplifying the breeding and facilitating seed production technology. The utilization of closed flower faba bean lines such as V23 and Vf70 available at IFAPA-Spain (Ana Torres in personal communication) might be useful for the development of highly autofertile lines and ease the maintenance of developed cultivars. Breeding for auto-fertility and the use of close flower lines would align with speed breeding approach described in the following paragraph.

7.6 Speed Breeding

The current breeding methods utilized in faba bean program of many national research systems are based on one filial generation per season which needs 8–9 years to develop new improved lines. The long breeding cycle to develop a new cultivar has negative impact on the value of genetic gains; and the improvement trends may not cope with the population growth and projected consumption in 2030. Therefore, there is a need to modernize the faba bean breeding programs and reduce the breeding cycle to accelerate the genetic gains. Strategy for reducing breeding cycle was introduced by Norman Borlaug in the 1950s at the International Centre for Maize and Wheat improvement (CIMMYT), which allowed growing two generations per year in Mexico (Ortiz-Monasterio et al. 2007). The faba bean program at ICARDA followed the same procedure by developing two generations per year using winter and summer season at Terbol station since 2012. This happened by doubling the number of generation by year using winter and summer season at Terbol station and by testing F_4 lines in lower latitudes in Egypt and in Sudan. This approach conducted to increase the annual genetic gains from an average of 0.8–1.5% per year (unpublished data). Reducing further the breeding cycle can be achieved through the implementation of speed breeding approach. Speed breeding (SB) increased generation turnover per year can be carried out in numerous ways, one of which involves extending the duration of plants' daily exposure to light, combined with early seed

harvest (Ghosh et al. 2018). Cold treatment (8/4 °C day/night for 2 days) after the onset of flowering induced the formation of more pods and faster pod set compared to the non-cold treatment (Mobini et al. 2020). Supplementary lighting is not therefore the only basis for rapid generation advance in plants. This approach is promising for legume crops in general and need to be tested in faba bean and can help to achieve 6.8 generations per year.

7.7 Genetic and Genomics Research

Except the first report of Erith (1930) on genetic inheritance, little research on faba bean genetics was developed in the following years (O'Sullivan and Angra 2016). Asynaptictrisomic mutants were identified and analyzed by Sjödin (1970, Sjödin 1971). This finding could lead to assign genetic markers to physical chromosomes (vaz Patto et al. 1999). Genes controlling rhizobia symbiosis and pigment composition were also identified (Duc and Picard 1986), with segregating recombinant inbred lines (RILs) being used to determine the Mendelian inheritance of a seed dormancy gene (Ramsay 1997).

Genomics research in faba bean is essential to improve the efficiency of the breeding programs. It addresses selection intensity and accuracy as it is expected to allow selection of large population at early stages. As other legume crops, faba bean improvement is currently impeded by a lack of genomic resources. The huge genome size (~13 Gb) of faba bean is the major limiting factor to achieve a comprehensive genomic information about this crop. Many reviews on genomic research in faba bean were recently published (O'Sullivan and Angra 2016; Webb et al. 2016; Maalouf et al. 2019).

The development of recombinant inbred lines (RILs) for the identification of associated molecular markers led to the production of high-resolution linkage maps and the identification of quantitative trait loci (QTLs) (Torres et al. 2010; O'Sullivan and Angra 2016). The linkage-based sequences were used to construct maps that are syntenic with other legumes (Webb et al. 2016). In a map of 687 V. *faba*, SNP markers assigned a linkage group, analogous to one of the six faba bean chromosomes. This was aligned with *M. truncatula* genomic regions and sequences to corresponding *V. faba* chromosomes (Webb et al. 2016). Torres et al. (2010) made the first review of the major achievement in marker-assisted selection (MAS). Recent studies focused on developing molecular markers for selecting to heat stress lead to the identification of simple sequence repeat (SSR) markers (Babiker et al. 2018) and SNP markers (Hu et al. 2018) associated with yield components under heat stress conditions. In other hand, Mohammed et al. (2013) identified gene for one of the putative QTL: SNP locus *Vf_Mt3 g086600* (associated with freezing tolerance). The genome sequences of *M. truncatula*, as well as of *Lotus japonicas*, *Glycine max*, *Lens culinaris,* and *Cicer arietinum* (Satovic et al. 2013; Varshney et al. 2014; Webb et al. 2016) became since then very important tools in faba bean research.

Ray and Georges (2010) were the first contributor to faba bean transcriptome obtained with the release of approximately 5,000 expressed sequence tags (EST) from developing embryos of the faba bean variety 'Windsor'. The expansion of transcriptome and genomic sequence data sets have allowed the development of huge numbers of markers, leading to significant increase in map coverage and marker density. The transcriptome research has also been used to assess the drought tolerance in faba bean, as i.e., the transcriptome profiling of the drought-tolerant faba bean cultivar Hassawi-2, under drought stress conditions using RNA sequencing (Khan et al. 2019). Khan et al. (2019) reported that 606.35 M high-quality pair-end clean reads yielded 164,679 unigenes of leaf tissues assembled. This finding can be used to improve drought tolerance in elite faba bean cultivars and to develop tolerant germplasm for other legume crops.

Proteomics research is applied to study the proteome changes under vernalization, chilling treatment and drought stress in several crop species including rice, maize and wheat (Yan et al. 2006; Rinalducci et al. 2011; Wang et al. 2016). In recent past, proteomic studies were also conducted in faba bean to characterize its response to vernalization (Cao et al. 2017) and drought stress (Li et al. 2018); The changes in proteome profile in faba bean subject to the vernalization were examined using the Isobaric Tag for Relative and Absolute Quantification (iTRAQ) LC–MS/MS approach to assess large-scale identification of vernalization-related proteins. In the other hand, Li et al. (2019) were the first to identify the abundance of proteins in faba bean under drought stress conditions. They suggested that proteins related to the cell defence pathways are modulated by overlapping signalling mechanisms, which provided information for overall understanding and engineering strategies to improve faba bean drought tolerance. These authors also confirmed that 25 proteins were clearly downregulated, and five proteins were upregulated in 30 differentially expressed proteins. Heat shock protein 81-2 stimulate new peptides further folds into a functional protein, assist the degradation of misfolded proteins to resist drought stress. The identified downregulated proteins (Li et al. 2019) mainly regulate the balance of stress defence, energy metabolism, cytoskeleton and oxidation, and the upregulated proteins can regulate proteins folding and aggregation and photosynthesis system.

ICARDA and Washington State University conducted genome-wide association studies (GWAS), where SNP marker calling program UNEAK (Universal Network Enabled Analysis Kit) found 10,950 variant loci from the sequence data. GWAS (Hu et al. 2018) and effective SNPs under under heat prone environments were identified (Maalouf et al. 2018). Genomic selection is an effective method in recurrent selection within synthetic populations (Müller et al. 2017), with more accurate predictions for single plants selection than for yield potential. Therefore, the genomic selection is useful for identifying superior individual plants. More recently, discovery of gene-based SNP markers for herbicide (Abou Kahter et al. in press) and heat markers (Maalouf et al. in press) and the construction of a high-density consensus map were achieved (Carrillo-Perdomo et al. 2020). Also, a new faba bean exome assembly originated from transcriptome data of four accessions (Hiverna, Nova Gradiska,

Silian and Quasar). These resulted in the identification of many SNPs of the most informative type due to their location in genes.

7.8 Conclusions and Future Direction

Faba bean crop remains one of the key important grain legumes specially for cool and temperate environments. The crop suffers from many abiotic stresses described briefly in this chapter such as drought, heat, and cold stresses as well as saline and acidic soils. These constraints are key limiting factors of the faba bean production. Due to limited funding in faba bean, the breeding programs are based mostly on field screening and therefore on the development of new cultivars required at least 8–10 years. The reduction of breeding cycle is possible as successfully demonstrated at ICARDA breeding program with maximum 5 years to reach new cultivars. In addition, modern speed breeding (SB) method allows development of 6 generations per year and is considered one of pioneer innovation that can lead to increase significantly genetic gains.

The future potential for the application of association studies will be useful for trait mapping, genetic diversity and linkage disequilibrium studies or map-based cloning and will enable faba bean MAS and genomic-assisted breeding as well as the identification of candidate genes of agronomic interest through synteny-based approaches. This will open the scope to identify markers associated with most economical traits and conduct precise screening in very large population size in early generation allowing the reduction of the breeding cycle and the increase selection of intensity which will be expressed in higher attainable genetic gains in faba bean.

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Chapter 8
Genomic Designing for Abiotic Stress Tolerance in Mungbean and Urdbean

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Abstract Mungbean and urdbean are two of the most important Asiatic *Vigna* species belonging to subgenus Ceratotrpis, which contains both cultivated and wild species. Asiatic *Vigna* species are distributed across Asia with rich diversity occuring in Southeast Asian regions. These *Vigna* species have huge economic importance in the region. These are consumed in the form of dal, curries, soup, sweets, and snacks. Mungbean and urdbean both are easily digestible protein source which takes care of protein needs of the vegetarian population. The germinated seeds have high nutritional value; the high lysine value makes both these crops an excellent complement to human nutrition, besides providing nutritive fodder to milch animals, as green manure, and cover crops. This review makes an effort to discuss different types of abiotic stresses affecting production, extent of losses caused, role of importance of *Vigna* pulses in genetic resources and diversity available in *Vigna* species, major milestones in Asiatic *Vigna* genetics, gene mapping, QTL mapping and recent developments in transgenics, comparative, and functional genomics related to different abiotic stresses.

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8.1 Introduction

The genus *Vigna* comprises more than 200 species, out of which only seven are of agronomic importance (Sai and Chidambaranathan 2019). Among these, mungbean and urdbean are cultivated since the prehistoric period specifically in the Asian countries (Singh et al. 2016a, b). Both the crops are warm temperate, or tropical in nature, valued for their grain containing high and easily digestible proteins. The seeds of these two crops contain about 25–28% protein, 1–1.5% oil, 3.5–4.5% fiber, and 62–65% of carbohydrates on a dry weight basis. In comparison to other pulses, the protein of mungbean and urdbean contains more concentration of lysine, therefore; it acts as an excellent source of nutrition that complements the cereals (Singh et al. 2016a, b). In addition to their nutritional values, mungbean and urdbean also play important role in sustaining soil fertility by fixing atmospheric nitrogen. It is observed that crops grown on 90% of arable land experience one or more stresses. Abiotic stresses cause more than 50% of crop loss worldwide (Rasool et al. 2013; Rodziewicz et al. 2014). Abiotic stress is a broad term that includes stresses like drought, waterlogging, salinity, heat, chilling, mineral toxicities, etc. One or more such stresses negatively affect the adaptability and yield of legumes. Mungbean and urdbean also suffer from several abiotic stresses. While based on multi-location trials and controlled environmental conditions, mungbean was found comparatively tolerant to drought, heat, waterlogging, frost, and temperature (Singh et al. 2016a, b). Global warming is leading to an increase in several kinds of abiotic stresses which limit crop production (Xiao et al. 2017). Drought and salinity are already widespread in many regions and are expected to cause serious salinization of more than 50% of all arable land by the year 2050 (Ashraf 1994). In this era where population growth exceeds food supply, plant breeding approaches aimed at overcoming severe environmental stresses need to be fully implemented. Plant adaptation to abiotic stresses is controlled by a cascade of gene networks. The genetically complex responses to abiotic stresses are multigenic and thus more difficult to control (Wang et al. 2003). For this reason, modern breeding approaches or biotechnology can be used for limiting abiotic stresses (Flowers 2004).

In the past few years with advancement in breeding science, different mechanisms have been utilized for abiotic stresses, such as:

1. ***Physiological and Biochemical***: Involve expression of plant defense-related enzymes to suppress overproduction of reactive oxygen species (Gill and Tuteja 2010).
2. ***Biotechnological***: Some of the recent investigations have shown that gene expressions that are highly involved in plant defense mechanisms encode proteins for abiotic stress tolerance. Several important achievements were obtained through in vitro genetic transformation to improve plant stress tolerance (Noman et al. 2017). Biotechnology approaches play a vital role in mining

candidate genes potentially involved in drought stress tolerance (Mishra et al. 2017).

3. ***Genomic Resources***: It can be a perfect method for investigating the genetically complex system for abiotic stress tolerance (Shen et al. 2018). Based on stability and response to abiotic stresses quantitative trait loci (QTL) can be categorized as, "adaptive and constitutive" (Collins et al. 2008). The adaptive QTLs can be detected under specific environmental conditions, which indicate that QTLs responsible for controlling different stresses.

8.1.1 Economic Importance

Mungbean or greengram (*Vigna radiata* L. Wilczek) and urdbean or blackgram (*Vigna mungo* L. Hepper) have a distinctive position in cropping systems because of short duration habits, diversified uses, and high per day productivity and are mainly cultivated in the Indian subcontinent and other Asian countries. On account of their duration and photoperiodism, they are considered excellent crops for crop diversification and intensification. These pulses are an integral part of cropping systems due to their short lifecycle and are of great significance in sustaining productivity in cereal-based agriculture by fixing atmospheric nitrogen through symbiotic association with Rhizobium bacteria (Parashar 2006; Snapp et al. 1998). These legume crops have the ability to add 30–40 kg N/ha to the soil from atmospheric nitrogen. They are an important and cheap source of proteins, fiber, antioxidants, and phytonutrients, vitamins, and minerals (Itoh et al. 2006). Mungbean is known to contain 24–28% protein, 1.0–1.5% fat, 3.5–4.5% fiber, 4.5–5.5% ash, and 59–65% carbohydrates on a dry weight basis (Tsou 1979) and provides 334–344 kcal energy (Srivastava and Ali 2004). Urdbean is also very nutritious as it contains high levels of protein (25%), potassium (0.98%), calcium (0.14%), iron (0.0076%) and niacin (0.0014/100g) (USDA database). It is also rich in vitamin A, B1, B3 and has a small amount of thiamine, riboflavin, niacin, vitamin C, and contains 78–80% nitrogen in the form of albumin and globulin (Das et al. 1998). They are most popular in developing countries by complementing meat protein components where meat is not dominant in the human diet. They are consumed as sprouts, as whole seeds, or split cooking and flour all over the world. Seeds can be ground into flour and used for making typical Indian flatbread called *papadum* (Jansen 2006). After pod picking, green plants and seed husk can be fed to the cattle. Mungbean crop is in high demand in the dairy industry as forage for producing high-quality meat and milk (Boelt et al. 2014). These crops are known to reduce the cost incurred on weed control by controlling weed flora up to 20–45% when intercropped with tall cereals (Ali 1988). These are also grown for green manure, cover crop, forage, and hay (Gohl 1982). But, mungbean makes good hay than urdbean.

8.1.2 Reductions in Yield and Quality Due to Abiotic Stresses

Static production of mungbean and urdbean in the last decades is largely caused by their susceptibility to several biotic and abiotic stresses at different growth stages of the crops. Among abiotic stresses, factors associated with water (drought and flooding); soil (nutrient deficiency, salinity, pesticide, and heavy metal pollutants, etc.), atmosphere (wind, cold, heat, and frost); and mechanical factors (soil compaction) (Hanumantha Rao et al. 2016) are major causes of low productivity. Over the decades, a drastic reduction in crop yields was observed because of changing climatic conditions (Boyer et al. 2013), which further adds to the complexity of plant environmental interactions (Goyary 2009). Drought and flooding are considered to be regular phenomenon in all grain legume growing areas. In mungbean, if drought stress occurs during the flowering time and podding time 31–57% and 26% yield losses are reported, respectively (Nadeem et al. 2019). It is evident from the study of Fathy et al. (2018) that, 30% moisture stress during the vegetative stage causes nearly 20% seed weight reduction however, at the reproductive stage seed yield losses go up to 50–60% in *Vigna* pulses. At the reproductive stage, yield reduction is due to flower abscission (Moradi et al. 2009), reduction in pod initiation, and pod growth (Begg 1980). At, temperature extremes plants are sensitive and might cause severe loss of productivity. Severe flower abortion and yield losses are noticed when the flowering stage coincides with high temperature (>45 °C). Each degree rise in temperatures above required could reduce the seed yield by 35–40% (Sharma et al. 2016). Salinity also affects the growth of the plant by mainly affecting biochemical mechanisms. It is expected that by mid of the 21st century, increased salinity will result in ∼50% loss of arable land (Hasanuzzaman et al. 2012). The salinity level of ∼50 mM NaCl can cause >60% yield losses in mungbean across the world (Abd-Alla et al. 1998). Preharvest sprouting yet one more abiotic stress which causes yield reduction up to an extent of 60–70% is reported to occur in mungbean (Durga and Kumar 1997).

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

United Nations report (2017) predicts world population would be ≈9.8 billion by 2050, therefore under changing climate scenario, meeting the food and nutritional security of growing population would be a daunting task. Increased production and consumption of pulses are important if global agriculture and food systems are to remain within planetary boundaries (Rawal and Cluff 2019). Pulses play a very important role in the sustainable intensification of crop production since they can be of dual-use, for human consumption and as cattle feed. Agronomic management of pulse crops is relatively easy with less input demanding nature, and comparatively climate resilient. Ecological services rendered by the pulses are of immense importance. The wealth of a nation depends on the health of its people, pulses provide nutritional

security to humans by providing a cheap source of quality protein, minerals, and micronutrients with low levels of cholesterol, and Glycemic Index contributes to the overall wellbeing of developing nations. Mungbean and urdbean due to their short duration and ability to fix nitrogen from the atmosphere limit the use of nitrogenous fertilizers derived from fossil fuels they help in mitigating the effects of climate change. Lack of crop rotation practices could be a serious threat to sustainable crop production since cereal–cereal cropping system will reduce total factor productivity (Gowda et al. 2015). The short duration nature of these crops helps to adopt well in multiple cropping systems and can provide desired sustainability to cereal-based cropping systems.

Though conventional crop improvement approaches have increased pulse production with the use of improved cultivars and scientific crop husbandry, the need of the hour is to breed for climate-smart pulse varieties tolerant to vagaries of climate. This helps in the expansion of mungbean and urdbean to newer ecological niches resulting in overall expansion of crop area leading to enhanced production making it more remunerative for smallholder farmers of developing nations.

8.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

Though traditional breeding has helped in developing several high-yielding varieties which perform well under optimum climatic conditions, most of them are susceptible to various abiotic stresses like heat, drought, waterlogging, and salinity. The global yield averages are 0.5 tons per hectares though 3 tons per hectares is considered achievable under favorable conditions (Douglas et al. 2020). Besides, that traditional breeding is time-consuming, laborious, and undesirable traits are transferred to offspring along with desirable traits from parents (linkage drag) during the hybridization program. To cope up with the climate change scenario and horizontal and vertical expansion of the varieties into new ecological niches requires exploiting the full genetic potential of the varieties released, which is possible when they are abiotic stress-tolerant. Abiotic stress tolerance is, governed by many traits which are controlled by polygenes, producing a range of phenotypes. The genetic gain obtained through traditional breeding by direct selection of traits affecting abiotic stress tolerance is less effective due to complex interactions within the plant and with the environment, affects the selection process, by making it ineffective due to low heritability. Selection intensity in traditional breeding depends on phenotypic selection, environmental variability, GxE interaction, and the error committed during the experiment adds complexity to phenotypic selection. Traditional breeding helps in improving one or few traits at a time, its efficiency is affected by a lot of factors as discussed above. Bringing all the favorable alleles governing beneficial traits, attributing to increased yield/quality is possible with the help of molecular

tools. Integrating phenotypic datasets, sequence and genotypic information, development of the marker-trait association and functional genomics approaches will help in developing ideal genotypes for changing climate scenarios.

8.2 Abiotic Stresses and Related Traits in Mung and Urdbean

8.2.1 Root Characters

The root is an indispensable organ of the plant for the absorption of nutrients and water by expanding its surface area and enhancement of explored soil volume (Hodge et al. 2009). Breeding for abiotic stress tolerance requires study/phenotyping of below soil-plant organs such as roots, to improve nutrient and water use efficiency. Genetic diversity study of root architecture is important as different types of plant root architecture explore the soil space effectively, thereby improving nutrient and water use efficiency under stressful conditions. Enhanced root-soil contact is known to increase Phosphorous efficiency (Panigrahy et al. 2009; Sarker and Karmoker, 2009; Lynch 2011). The presence of very fine roots (<0.5 mm diameter) and fine roots (0.5–2.0 mm) accounts for substantial portion of root system which is important for nutrient and water uptake (Liu et al. 2010; Zobel and Waisel, 2010; Liu et al. 2018). Root length is an important trait against drought stress; in general, variety with longer root growth has the ability to resist drought (Leishman and Westoby 1994; Kaydan and Yagmur 2008) Pandey et al. (2014) reported that root traits such as primary root length (PRL), total root length (TRL), total root surface area (TSA), total root volume (TRV), root average diameter (RAD), total root tips (TRT), root forks (RF), root surface area, volume, biomass, and root carboxylate exudation capacity were significantly higher in P efficient mungbean genotype compared to inefficient genotype. Traits such as root length, root volume, surface area, and the number of lateral roots contribute significantly towards P uptake at 45 days after sowing in urbdean (Jakkeral et al. 2009).

8.2.2 Root Phenotyping

Changing the architectural design of the root system may enhance desirable agronomic attributes such as yield, drought tolerance, and nutrient deficiency tolerance (Tuberosa et al. 2002; Beebe et al. 2006; Ghanem et al. 2011). Inaccurate phenotyping and limited population size in mapping may impede the use of genomics to improve root attributes in breeding programs (de Dorlodot et al. 2007). For translating current physiological and genetic breakthroughs into increased yield and productivity, particularly in dry ecosystems, precise phenotyping and evaluation of root-related traits

are essential. Selection and breeding of cultivars with root systems that use nutrients and water more efficiently than current varieties is an effective strategy for growing edaphic stress adaptation (Siddique et al. 2001). Several phenotyping techniques have been reported for examining root features, including hydroponic systems using growth bags or germination sheets (Bonser et al. 1996; Atkinson et al. 2015; Wasson et al. 2017), agar and aeroponic systems (Liao et al. 2004), soil rhizotrons (Manschadi et al. 2008; Gregory et al. 2009; Wu et al. 2018), deep column methods (Wiese et al., 2005), transparent containers (Narayanan et al. 2014), PVC pipes (columns), and glass-walled soil-filled rhizoboxes, but these methods are expensive and time-consuming. The use of digital imaging and software methods to evaluate root images is an innovative and efficient way to accurately assess root traits (Palta et al. 2007; Richard et al. 2015; Figueroa-Bustos et al. 2018). A variety of software programs can be used to derive two-dimensional root morphology traits. This is in contrast to DART (Palta et al. 2007), a commercially accessible root analysis tool WinRhizo™ (Regent Instruments, Quebec, Canada) and EzRhizo (Figueroa-Bustos et al. 2018) a free, fully integrated, and automated SmartRoot (Chen et al. 2011) platform for small root systems.

8.2.3 Drought Tolerance

In the global climate change scenario, food shortage, water scarcity, malnutrition, and population growth are some of the major challenges faced by mankind. Drought stress is one of the major threats faced by the agriculture sector all over the world. The deficiency of precipitation over an extended period can simply be termed drought. Drought stress poses a great threat to agricultural productivity. Drought stress is one of the most complex and least-understood abiotic stresses. Drought stress can be characterized based on intensity/severity, duration, and spatial extent. The intensity/severity of drought stress is predicted to increase in near future. Most of the legume crops are sensitive to drought stress, especially at vegetative and reproductive stages, since they are mostly grown in dry areas. Drought stress causes remarkable yield losses. Prominent symptoms of drought stress include reduced germination, stunted growth, hampered photosynthetic apparatus, reduced net photosynthesis, and reduction in nutrient uptake process (Nadeem et al. 2019). It is very important to understand the agronomic and genetic basis of the drought stress for sustainable ecological, economical, and agricultural management for breeding drought-tolerant legume crops with higher water use efficiency.

Mungbean and urdbean yield are largely static due to crop's susceptibility to various biotic and abiotic stresses among which drought stress is one of the major limiting factors (Sehrawat et al. 2013). Mungbean is predominantly cultivated in India under rainfed cultivation, so the crop can be affected by drought stress in its short growing period. Mungbean is also cultivated as a spring/summer crop in the rice-wheat cropping system or other short-duration oilseed crops or vegetables

including peas after harvest of wheat. Summer mungbean is highly sensitive to moisture stress during the month of May and June when the temperature goes up to 46 °C in Western Indo-Gangetic plains. Scarcity in irrigation water and intense heatwave during May–June coinciding with the reproductive phase causes yield reduction, due to terminal drought usually coinciding with late reproductive stage, causing a significant reduction in seed yield due to pollen inviability, lack of fertilization and thus resulting in complete flower shedding. During the *Kharif* or rainy season, crops are affected by intermittent drought, which may occur anytime during vegetative growth, due to a break in rainfall or insufficient rains at the vegetative stage leading to poor establishment affecting the total seed yield. Drought stress can affect prospects of horizontal and vertical expansion of mungbean and urdbean, hence understanding the genetic/physiological basis for drought tolerance in these crops are essential for breeding climate-smart drought-tolerant cultivars. In this regard, many workers have attempted to understand the basis of drought stress in these crops. Singh et al. (1999) have ranked warm-season food legumes in increasing order of drought resistance as soybean, followed by urdbean, mungbean, groundnut, Bambara groundnut, lablab bean, and cowpea. Few workers have screened mungbean germplasm, for drought tolerance (Parameswarappa and Lamani 2003; Varma and Garg 2003). Studies on genotypic differences in physiological traits and dry matter partitioning in mungbean were conducted to measure the association of traits with crop performance under drought conditions (Kumar and Sharma 2009). Studies were also conducted to decipher the response of mungbean for the water stress at three different growth stages; three weeks after planting (3 WAP), 6 WAP, and 8 WAP. The study revealed water stress significantly affects each of the measured parameters at 6 WAP, the flowering and pod filling stage of mungbean (Ranawake et al. 2011).

Good establishment of the seedling stage is very important for getting a higher yield of the crop and so seedling stage drought stress tolerances in mungbean germplasm accessions were studied (Tripathy et al. 2016; Mandi et al. 2018). Dutta et al. (2016) studied quick and reliable physiological marker for screening for drought susceptibility, through evaluation of physiological and biochemical changes in leaves during seedling stage to aid in future genetic manipulations for establishing drought tolerance in the crop. Pezhman et al. (2016) studied physiological responses of mungbean when vegetative growth stage was exposed to drought stress and observed significant decrease yield and yield components. Eswaran and Anbanandan (2018) studied the genetics of drought tolerance in mungbean, their study revealed the importance of both additive and nonadditive genetic variance in the inheritance of traits. Sairekha and Mohan Reddy (2017) conducted path analysis to find out the direct and indirect effects of morphological and drought-related traits on seed yield in mungbean. Liu et al. (2017) constructed a novel genetic linkage map using simple sequence repeat (SSR) markers and stable quantitative trait loci (QTLs) were identified for six drought tolerance-related-traits. Ali et al. (2018) examined the drought tolerance of various mungbean cultivars/lines based on the seed germination characteristics in relation to the seedling's antioxidative potential and nutrient uptake. Manu et al. (2021) have studied response of mungbean germplasm lines under drought and heat stress conditions in spring/summer season, and role of physiological traits such as

SPAD chlorophyll meter reading (SCMR), and Light response of chlorophyll fluorescence derived photosynthetic electron transport (ETR) in the chloroplasts has been studied. The morpho-physiological and molecular screening and the cultivar identification are establishing the theoretical and practical foundation for drought-tolerance breeding of mungbean and urdbean.

8.2.4 Heat Tolerance

Heat stress has become a significant threat to food security as global warming progresses. It was predicted that global air temperature is to rise by 0.2 °C per decade, which can result in temperatures 1.8–4.0 °C beyond this level by 2100 (Intergovernmental Panel on temperature change 2007). Pulses show varying degrees of sensitivity to heat, which reduces their potential performance at different developmental stages like germination, seedling emergence, vegetative phase, flowering, and pod/seed filling phase (Bhandari et al. 2016). To deal with ever-changing temperature extremes, researchers are working to generate resistant genotypes in legumes using traditional breeding procedures and/or, more recently, molecular breeding approaches.

Mungbean and urdbean are grown over a wide range of soils and agroclimatic zones of the world. Despite their importance in soil and human health, their true yield potential has not been achieved due to the risk of several abiotic stresses. High-temperature stress during germination and flowering causes significant yield losses in both these crops. Terminal heat stress could lead to considerable flower drop and thus reduced pod set. The terminal heat stress is a severe problem in India, more particularly in spring and summer mungbean, if the temperature is over >40 °C then there's the reduction in seed yield because of pollen inviability, lack of fertilization and flower shedding. To increase the mungbean production it is important to develop mungbean genotypes that can retain the maximum number of flowers and produce productive pods during high temperatures (>40 °C) (Singh and Singh 2011). Heat stress affects the assorted molecular and physiological processes related to the growth, development, and economic yield of a crop (Begg and Turner 1976). Plants respond to heat stress by activating complex molecular networks, like signal transduction, metabolite production, and the production of heat stress-proteins. Heat stress tolerance is a polygenic trait influenced by a number of genes, transcription factors, proteins, and hormones. Therefore, to enhance heat stress tolerance; a sound knowledge of varied mechanisms involved in the response to heat stress is required. Although the heat stress is one of the important abiotic stresses which is a major constraint in increasing the productivity of these crops, only limited work has been carried out on breeding for tolerance to abiotic stresses. During this era of genomics, next-generation sequencing techniques, availability of genome sequences, and advanced biotechnological tools open several windows of opportunities to enhance heat stress tolerance in crop plants.

Several workers have evaluated mungbean genotypes for heat tolerance. Kumari and Verma (1983) showed that high-temperature stress has negative affect on flower

retention and, as a result, pod formation is hampered. Flower shedding in mungbean is highly prevalent due to high temperatures, and the extent of flower shedding has been observed as high as 79% (Kumari and Verma 1983). Khattak et al. (2006) evaluated mungbean genotypes against high temperature and reported that none of the genotypes showed absolute tolerance to flower shedding under high temperature (>40 °C). From observations of the International Mungbean Nurseries, Poehlman (1978) suggested that a mean temperature of 28–30 °C is optimum for this crop and lower temperature (<20 °C) reduced germination and affects the expansion of the plant. Increased flower shedding under high temperature during flowering periods in legumes have been reported by different researchers (Tickoo et al. 1996; Rainey and Griffiths 2005). Das and Mukherji (1994) reported that total sugar content, proline level increased during high-temperature stress, and starch content reduced with high temperature. It was reported that heat shock increased sugar content by activation of the starch degrading enzyme and that the increase in proline content maybe because of high-temperature stress. Rainey and Griffiths (2005) reported the abscission of reproductive organs as the primary determinant of yield under heat stress in many annual grain legumes. In subtropics or at higher altitudes, mungbean is sometimes planted when mean night temperatures are but 20 °C, in such condition germination is delayed and reduced. Alagu et al. (2015) studied the effect of heat stress during the reproductive stage based on yield attributing traits and stress indices in 20 mungbean cultivars grown in different agroclimatic zones of India. Among the cultivars studied three cultivars such as Samrat, IPM-02-3, IPM-2-14 were identified as heat-tolerant as indicated by their low heat susceptibility index.

Kaur et al. (2015) elucidated the response of mungbean genotypes to heat stress with regard tore productive biology, leaf function, and yield traits. Two genotypes (SML 832 and 668) were subjected to HTs (>40/25 °C; day/night) during the reproductive stage. A drastic reduction in pod set, number of filled pods (32–38%), seed number (43–47%), and seed yield (35–47%) was observed with no or less effect on phenology, flowering duration, and podding. SML 668 was found to be more sensitive to heat stress than SML 832.

Bindumadhava et al. (2018) based on physiological traits identified heat-tolerant lines viz., EC-693357, EC-693359, Harsha, and ML-1299 which could be utilized in mungbean breeding programs to develop climate-resilient varieties. Similarly, Sunayana et al. (2017) identified high-temperature tolerant genotypes of mungbean such as MH-805, MH-736, MH-421, IPM-02-03, MH-721, MH-810, IPM-409-4, and Ganga-8, based on morphological and physiological traits, further Sunayana et al. (2017) concluded that genotypes Pusa 105, M 395, EC 393410, and Pusa Vishal had good membrane stability index and canopy temperature difference but lower yield, therefore, these genotypes can be utilized in the future breeding program for the development of high temperature tolerant and drought tolerant genotypes.

Basu et al. (2019) evaluated 116 mungbean genotypes for flowering, biomass, and yield attributes. Based on heat shock treatment (37–52 °C) and recovery assessed at 30 °C, they identified EC 398889 and LGG 460 as heat-tolerant and heat-sensitive genotypes, respectively. They also studied pollen germination and SuSy activity light-temperature response of photosynthesis, chlorophyll fluorescence imaging of

quantum yield (Fv/Fm), and electron transport rate (ETR) between heat-tolerant (EC 398889) and heat-sensitive (LGG 460) genotypes. Eleven SSR markers were used for molecular profiling of selected accessions, and the markers CEDG147, CEDG247, and CEDG044 distinguished tolerant and sensitive groups of accessions.

8.2.5 Salinity Tolerance

Soil salinity has been a problem in agriculture for over 3000 years in some areas of the world (Flowers 2006) and it has been exacerbated by irrigation water sourced by surface irrigation in arid and semi-arid environments (Hanumantharao et al. 2016). Due to increased use of low quality water for irrigation and soil salinization, salinity is a major abiotic stress restricting plant growth and productivity in many parts of the world. Complex physiological characteristics, metabolic pathways, and molecular or gene networks are all involved in plant adaptation or resistance to salinity stress. For the production of salt-tolerant plant varieties in salt-affected areas, a detailed understanding of how plants react to salinity stress at various levels, as well as an integrated approach combining molecular tools with physiological and biochemical techniques are needed. While the mechanisms underlying salinity tolerance are far from being fully understood, recent studies have identified numerous adaptive responses to salinity stress at the genetic, cellular, metabolic, and physiological levels.

Legumes are salt-sensitive crops, and in water-scarce environments, a high concentration of Na^+ and Cl^- ions around the root zone restricts the geographical range of legumes in arid and semiarid climates where evapotranspiration exceeds precipitation. Plants are usually affected by salinity in two ways: osmotic stress and ion toxicity. However, there is a third mode that affects legume species in particular: decreased nodulation by rhizobia. However, the response of legumes and other plant species varies depending on the climate and the severity of stress.

Salt stress affects seed germination (Rahman et al. 2000; Dash and Panda 2001; Al-Moaikal 2006; Sangeetha and Subramani 2014) fresh and dry biomass, shoot and root length, and yield attributes in most crops (Promila and Kumar 2000; Rabie 2005; Ahmed 2009). Reduction in germination is due to low osmotic potential that prevents water absorption or by causing toxic effects from Na^+ and Cl^- ions (Khajeh-Hosseini et al. 2003), due to the specific ion effect (Hassen 1999). Salt stress reduces nutrient uptake and distribution by affecting root growth and elongation. Naher and Alam (2010) undertook screening of mungbean varieties at higher NaCl concentrations reported that the BARI Mung4 has performed better with increased nodule size, however the number of nodules per plant decreased as salinity increased. Salinity tolerance is a genotype-dependent and growth stage-specific phenomenon in nature, so tolerance at an early (seedling) stage does not mean tolerance at later (maturity) stages (Sehrawat et al. 2013). It also requires multidimensional responses in plants at different organ levels (e.g., tissue, genetic, physiological, and plant canopy)

(Hanumantharao et al. 2016). Over the years, little progress has been made in developing salt-tolerant mungbean varieties due to this difficulty and a lack of sufficient introgression techniques (Ambede et al. 2012; Hanumantharao et al. 2016).

In many crops (barley, rice, pearl millet, maize, sorghum, alfalfa, and many grass species) lot of work has been done to improve salt tolerance. Butin legumes, in general, and mungbean in particular very few studies can be found. In breeding program, rapid screening methods are needed to identify putative donor parents (Saha et al. 2010). Manasa et al. (2017) used the Salinity Induction Response (SIR) technique to screen 40 mungbean lines sourced from the World Vegetable Center for salinity tolerance at the seedling and whole plant levels by canopy phenotyping assay under 150 and 300 mM NaCl stress scenarios. Both tolerant and susceptible lines showed a significant reduction in growth and yield parameters, but a few lines (EC 693357, 58, 66, 71, and ML1299) showed comparable biomass and pod yield to non-stressed control plants. One of the reasons for tolerant lines, tolerance may be their inherent ability to portion salt to vacuoles (more influx of Na^+ ions) during high salt concentrations in the cytocol. Shabina and Mehar (2011) found Punt Mungbean to be more salt tolerant, with higher net photosynthesis, plant dry mass, and seed yield, all of which are linked to low Na^+ and Cl^- content and high osmolyte accumulation in the plant leaves. While, cultivar T44 suffered the most damage and was identified as a vulnerable cultivar. Few cultural methods have been reported to mitigate damage by salt stress. Selenium at a low dose effectively reduced salt damage in urdbean by inhibiting Na^+ uptake and improving antioxidant defence role for sucrose and decreasing sugar accumulation (Hassan et al. 2020). The application of gibberellic acid (GA) alleviates the adverse effect of salinity on urdbean seedlings and can be attempted in the field trials (Dheeba et al. 2015). Hasan et al. (2017) reported yield and yield traits of mungbean were more affected than in urdbean and identified urdbean (BARI Mash-1) which is relatively more salt tolerant than mungbean (BARI Mung-5) based on morphological and yield parameters. Some *Vigna* crops were tolerant to salt stress, such as cowpea, Bambara groundnut (*Vigna subterranea*), zombi pea [*Vigna vexillata* (L.) A. Rich], and urdbean. Whereas, azuki bean, rice bean, mungbean, and moth bean were susceptible, the tolerant crops might be directly applied to cultivation in moderately saline soils (Iseki et al. 2016).

8.2.6 Cold Tolerance

Among various stresses, cold temperature causes considerable yield losses in pulse crops by arresting the growth of the plant. Cold temperature stress results in reduced germination, stunted growth, yellowing, pollen sterility, delayed heading, and reduction in grain yield of crops (Suzuki et al. 2008). The main mechanism of cold injury is that it affects dark reaction in chloroplast consequently, reduces supply of $NADP^+$ to photosystem II which, promotes over-production of reactive oxygen species (ROS) like superoxides, hydrogen peroxides, hydroxyl radicals in the cells (Hung et al. 2005). Low temperatures in combination with high light intensity lead to serious

cellular damage in the plants due to photo-oxidation (Allen and ort 2001). Such type of physiological and biochemical dysfunctions are commonly noticed in tropical and sub-tropical crop plants when, they are exposed to below 10 or 12 °C (Graham and Patterson 1982). These alterations include lipid composition and membrane structure of the cell (Lyons and Raison 1970), metabolic modifications (Levitt 1980), redistribution of intracellular calcium ions (Bush 1995), cellular leakage of amino acids and electrolytes, and diversion of an electron to the alternate pathway (Leopold and Musgrave 1979), change in cellular protein composition, enzymatic activity and phosphorylation of thylakoid proteins (Bannett 1991). Low temperatures stress on urdbeanis reported to have a detrimental effect on its production and productivity (Eapen 2008). Chang et al. (2001) reported that exposure of the mung bean seedlings at 4 °C for 2 days can result in irreversible chilling injury by electrolyte leakage. Amelioration of chilling injury by exogenous application chemicals like choline, reduced chill-sensitivity in seedlings of mung bean, by increasing the concentration of chlorophylls, carotenoid, and the carotenoid/chlorophyll ratio (Guye et al. 1987). Application of paclobutrazol, abscisic acid, and hydrogen peroxide resulted in alleviation of chilling injury in mungbean by enhancing the free radical scavenging system (Saleh 2007). Lawn et al. (1988) reported that *Vigna radiate* var. *sublobata* species are tolerant to low temperature which can be used as donor for transferring cold tolerance.

8.2.7 Flooding and Submergence Tolerance

Waterlogging is one the major abiotic stresses that affects growth and yield of mungbean and urdbean in the tropical and subtropical regions of the world. Fernandez and Shanmugasundaram (1988) have inferred that cultivation of mungbean where the annual rainfall is >1000 mm may incur severe yield losses.

Mungbean cannot withstand waterlogging particularly during the early stages of crop growth (Tickoo et al. 2006). It causes serious damages to germination and emergence (Ullah 2006). Waterlogging during crop stand affects closing of stomata, resulting in increased concentration of ethylene and decreased activity of rubisco enzyme leading to reduced crop growth rate (CGR), net assimilation rate (NAR), and leaf expansion rate (LER) of plants. Flooding reduces aeration to roots thereby reducing nodule activity and nitrogen fixation (Singh and Singh 2011). Waterlogging also restricts root and shoot growth which may result in the total loss of crop yield (Toker and Mutlu 2011; Islam 2016). Plants surviving water logging may further get attacked by fungal diseases and insect pest (Tickoo et al. 2006). Many workers have reported substantial yield lossin mungbean (Normile 2008; Kumar et al. 2013; Amin et al. 2016), depending on the growth stages encountering waterlogging.

A wide range of variation for waterlogging stress has been observed in mungbean (Islam et al. 2007) and urdbean (Rana et al. 2019a, b). Amin et al. (2015) evaluated mungbean genotypes for tolerance to waterlogging by maintaining 3–5 cm standing water at 24 days after emergence. They observed that the days to flowering and

maturity delayed in flooded plants over control depending on the genotypes. There was a significant reduction of total drymatter (TDM), number of pods per plant, seed size and seed yield of the mungbean genotypes compared to control.

Islam et al. (2019) reported application of nitrogenous fertilizer post-waterlogging improves waterlogging tolerance in mungbean. Under waterlogged conditions accumulation of ethylene plays an important role in flooding-induced adventitious root formation (Visser et al. 1996). Kumar et al. (2013) have reported proliferation of adventitious root in mungbean genotypes tolerant to waterlogging. Raina et al. (2019a, b) observed significant variations in ethylene sensitivity among mungbean genotypes exposed to waterlogging. The study also reveals a genotype-dependent transcriptional regulation of ethylene biosynthetic/responsive genes. Rameshreddy et al. (2019) screened 40 mungbean lines in specially designed 'Field Root Structures' to screen for waterlogging tolerance. Waterlogging treatment was enforced at three stages, viz., 30 days after sowing (30 DAS) for five days, at flowering stage (43 DAS), and at 60 DAS. Based on seed yield and total biomass (TDM) they classified AVMU 1001, AVMU 1201, VO 6381A-G, KPS-1, ML 1628, PDM 139, IPM 02-14 as waterlogging tolerant genotypes. Shibly et al. (2020) evaluated yield and yield-related traits for waterlogging tolerance in mungbean genotypes, and reported IPSA-10 and VC 6379 (23-11) as tolerant to waterlogging.

8.2.8 Other Abiotic Stresses

Tropical crops like mungbean and urdbean require hot and dry climate. Cloudy weather, continuous and heavy rains, adversely affect the flowering and podding, causing low yields. Pre-harvest sprouting (PHS) and pod shattering are also some of the factors hampering the production of these crops under climate change scenario. PHS is sometimes referred to as weather damage. The phenomenon of germination of seeds in the pod, usually under wet conditions shortly before harvest, is termed pre-harvest sprouting (PHS). Warm humid conditions at maturity in tropical and temperate regions are conducive to pre-harvest sprouting. PHS results in rupture of seed coat which is the physical barrier that protects the seed from adverse environmental conditions and disease-causing organism, lead to a reduction in seed quality and quantity (Ahmad et al. 2014).

Among the legumes, the incidence of PHS is very high in *Vigna* species. Cultivars with prolonged flowering and pod sets are still prone to pod shattering, resulting in yield loss. A yield loss due to PHS is as high as 60–70% (Durga and Kumar 1997). Lens or strophiole In *Vigna* species regulates the entry of water inside the seed (Kikuchi et al. 2006). Preharvest sprouting can be mitigated by the incorporation of hardseededness in the cultivated variety (Humphry et al. 2005). Isemura et al. (2012) have mapped QTLs regulating water absorption in mungbean, azuki bean (Kaga et al. 2008) and in rice bean (Saravanakumar et al. 2004). Pod shattering is one of the ways of seed dispersal mechanism observed in wild species. Wild species of

Vigna are pod-shattering type, while most of the present-day cultivars are shattering resistant and have synchronous maturity.

8.3 Traditional Breeding for Abiotic Stress Tolerance

Improved varieties of mungbean and urdbean developed through traditional breeding methods have contributed immensely to maximizing legume productivity. Traditional breeding efforts started with the selection of superior high-yielding lines from locally adopted germplasm (Douglas et al. 2020) which were indirectly selected for different abiotic stresses. With the hope of increasing genetic gain more through increased selection efficiency, further studies were conducted on genetics of traits, floral biology, plant type and growth habit, pigmentation in different plant parts, leaf, stem, flower, pod seed, photoperiod response, yield traits and study of biotic and abiotic stress resistance. Various traditional plant breeding methods have been practiced for the genetic improvement of mungbean and urdbean. Ranali and cubero (1997) have discussed the basis of genetic improvement, through plant introduction, hybridization, early generation selection, mutation, and use of molecular markers. Dikshit et al. (2020) have discussed in detail about the role of plant introduction and methods like pure line breeding, recombination breeding, and mutagenesis. List of popular varieties and breeding method adopted in mungbean and urdbean has been given in Table 8.1.

Traditional methods involve scoring of phenotype which is environment-dependent and is neither stable nor reproducible. These phenotypes are very limited in numbers and without progeny test, it is impossible to distinguish between heterozygous from homozygous individuals. Phenotyping is time consuming and labor intensive (Table 8.2). Modern molecular mapping tools are independent of environmental influence. The use of molecular markers results in the construction of high-density linkage maps which help in mapping QTLs for quantitative traits. Molecular markers find applications in marker-assisted selection, marker-assisted recurrent selection, marker-assisted backcrossing, forward breeding, haplotype-based breeding, genomic selection, gene pyramiding, and comparative gene mapping for gene tagging. They are amenable for high throughput, covering the whole genome (Pictures 8.1 and 8.2).

8.4 Genetic Resources of Resistance Genes

8.4.1 Available Germplasms

The genus *Vigna* consists of several important species which can be a potential source of resistance to different abiotic stresses. Genus *Vigna* is divided into five subgenera: *Ceratotropis, Haydonia, Lasiospron, Plectrotropis*, and *Vigna*. Three of the subgenera

Table 8.1 Popular varieties and breeding methods adopted in mungbean and urdbean

S. No.	Approach/method	Varieties	Country	References
1	Introduction (as a variety/as a new variety after selection/as a parent in hybridization)	Pusa 105, Pusa 9531, Pant Moong 5, Pusa Vishal and SML 668 (Mung)	India	Dikshit et al. (2020)
		VC1973A and VC2778A (Mung)	China, Thialand	Srinives (1996)
		NM92, NM94 (Mung)	Pakistan	Ali et al. (1997)
		AVMU 0801, AVMU 1003 AVMU 8501 (Mung)	Kenya	Karimi et al. (2019)
2	Hybridization (for combining desirable traits)	Mung: Pant M 4 HUM 1, Meha, Shika, Virat IPM 02-3, IPm 2-14, PM6, IPM 99-125 and Pusa Bold 2, IPM 410-3, DGGV-2 Urd: KU 1, Narendra Urd 1, WBG 26, IPU 94-1, KU 300, LBG 17, Pant U 35, massh118, Vamban 7, TU 40, KU 301, Pant U-31, Pant U-40 IPU 02-43, KU 96-3, TAU 1, LBG 752, KU 300 and Uttara	India	Dikshit et al. (2020) Douglas et al. (2020) Singh et al. (2016a, b)
3	Mutagenesis	Mung: Pusa Vishal SML668, Pant Moong 2, MUM 2, Co 4, LGG 407, LGG 405 and BM 4, LGG-450 (Pushkara)	India	Dikshit et al. (2020) Singh et al. (2016a, b)
		NM92 and NM98	Pakistan	Dikshit et al. (2020)
		Chai Nat 72	Thailand	Dikshit et al. (2020)
		Urd: Prasad and Ujala, Vamban-2 (Drought tolerant) Prasad (B 3-8-8) Ujala (OBG-17)	India	Singh et al. (2016a, b)

Table 8.2 Different gene pools of mungbean and urdbean

Crop	Primary gene pool	Secondary gene pool	Tertiary gene pool	References
1 Mungbean	*Vigna radiata* var. *radiata*	*V. mungo* var. *mungo*	*V. angularis*	Chandel and Laster (1991) Dana and Karmakar (1990) Smartt (1981, 1985) Kumar et al. (2004)
	V. radiata var. *sublobata*	*V. mungo* var. *silvestris*	*V dalzelliana*	
	V. radiata var. *setulosa*	*V. aconitifolia*	*V. glabrescens*	
		V. trilobata	*V. grandis*	
		V. subramaniana	*V. umbellata*	
		V. grandiflora	*V. vexillata*	
		V. stipulacea		Tomooka et al. (2011)
		V. tenuicaulis		
		V. umbellata		
2 Urdbean	*V. mungo* var. *mungo*	*V radiata* var. *radiata*	*V. angularis*	Chandel and Laster (1991), Dana and Karmakar (1990), Kumar et al. (2004)
	V. mungo var. *silvestris*	*V. radiata* var. *sublobata*	*V. dalzelliana*	
		V. radiata var. *setulosa*	*V. glabrescens*	
		V. aconitifolia	*V. grandis*	
		V. trilobata	*V. vexillata*	

Source Kumar et al. (2011), Tomooka et al. (2011)

Picture 8.1 Screening of mungbean germplasm lines for drought tolerance at ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh India

Picture 8.2 Screening of urdbean germplasm lines for drought tolerance in rainout shelter at ICAR-Indian Institute of Pulses Research, Regional center, Dharwad, Karnataka, India

include crop species: *Plectrotropis* and *Vigna* have originated in Africa, and *Ceratotropis*, which originated in Asia, includes mungbean and urdbean. There are 21 species in the subgenus *Ceratotropis* (Tomooka et al. 2002b). Six new species have been included in the subgenus described from India (Dixit et al. 2011; Aitawade et al. 2012; Gaikwad et al. 2014, 2015; Latha et al. 2014; Balan et al. 2017). However, Takahashi et al. (2016) accepted *Vigna indica* and *Vigna sahyadriana* as distinct species based on DNA sequences and listed 23 species in subgenus *Ceratotropis* as 'validated'. The subgenus *Ceratotropis*has been divided into three subsections namely; *Aconitifoliae*, *Ceratotropis*, and *Angulares*. Three of the remaining four recently described species, *Vigna sathishiana*, *Vigna konkanensis*, and *Vigna pandeyana*, are classified in section *Ceratotropis*, and the fourth, *Vigna yadavii*, is classified in section *Angulares* based on morphology.

Potential variation in wild species for abiotic stresses has been mentioned in Table 8.3. Promising trait-specific germplasm of mungbean, urdbean and moth bean is mentioned in Table 8.4 and sources of resistance for various abiotic stresses in mungbean/urdbean is mentioned in Table 8.5.

Major Institutions Where Genetic Resources of Pulses are Maintained

- Global Gateway to Genetic Resources (GENESYS) (https://www.genesys-pgr.org/)
- World Vegetable Center (AVRDC), Taiwan (http://www.avrdc.org)
- Australian Temperate Field Crops Collection, Australia (http://agriculture.vic.gov.au)
- Banco de Germoplasma – Departamento de Recursos Genéticos e Melhoramento; Estação Agronómica

Table 8.3 Potential variation in wild *vigna* species for abiotic stresses

Character	Species	References
High photosynthetic efficiency and drought tolerance	*V. radiata* var. *sublobata*	Ignacimuthu and Babu (1987)
Drought tolerance	*V. aconitifolia*	Jain and Mehra (1980)
Heat tolerance	*V. aconitifolia*	Tomooka et al. (2001)
Heat tolerance	*V. riukinensis*	Egawa et al. (1999)
High tolerance to saline and Alkaline soils	*V. radiata* var. *sublobata*	Lawn et al. (1988)
Photo-thermo insensitivity	*V. umbellata* *V. glabrescens*	Pratap et al. (2014b)

Source Pratap et al. (2014a, b)

- Nacional, Instituto Nacional de Investigaçã Agrária, Portugal (https://www.genesys-pgr.org/wiews/PRT005)
- Centro de Investigación Agraria Finca La Orden – Valdesequer, Spain (https://www.genesys-pgr.org/wiews/ESP010)
- Centro Internacional de Agricultura Tropica (CIAT), Colombia (http://www.ciat.cgiar.org)
- Crop Germplasm Resources Information System, China (www.cgris.net/cgris_english.html)
- Crop Germplasm Resources Platform, Ministry of Science and Technology, China
- Institute of Crop Sciences, Chinese Academy of Agricultural Science, China (http://www.cgris.net/cgris_english.html)
- International Center for Agricultural Research in the Dry Areas (ICARDA), Syria (http://www.icarda.cgiar.org)
- International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India (http://www.icrisat.org)
- International Institute of Tropical Agriculture (IITA), Nigeria (http://www.iita.org)
- International Livestock Research Institute (ILRI), Ethiopia (http://www.ilri.cgiar.org)
- Institut National de la Recherche Agronomique, France (https://urgi.versailles.inra.fr/siregal/siregal/grc.do)
- Junta de Extremadura. Dirección General de Ciencia y Tecnología, Spain (http://centrodeinvestigacionlaorden.es)
- Leibniz Institute of Plant Genetics and Crop Plant Research, Germany (http://www.ipk-gatersleben.de)
- N.I. Vavilov Research Institute of Plant Industry, Russia (http://www.vir.nw.ru)
- National Bureau of Plant Genetic Resources, India (http://www.nbpgr.ernet.in)
- National Plant Germplasm System, USA (http://www.ars-grin.gov/npgs/index.html)
- NIAS Genebank, Japan (https://www.gene.affrc.go.jp/databases_en.php)

Table 8.4 Promising trait-specific germplasm of mungbean, urdbean and mothbean

Crop	Trait(s)	Accession(s)	Country of origin
Mungbean	Wide adaptability, earliness	EC 118889, EC 118894, EC 118895, EC 162584, EC 158782, EC 159734	Taiwan
	Tolerance to drought, flood, photoperiod insensitivity	EC 318985–319057	Taiwan
	High yielding	EC 391170-75	Indonesia
	Large seeded, long podded with shiny green seed coat	EC 393407-10	Bangladesh
	Heat tolerant, short and long duration	EC 397138, EC 396394–396423	Thailand
	High yielding	EC 390990-93	Taiwan
	High yielding	EC 428862	Nepal
	Early maturity	EC 512780-793	USA
	Heat tolerant	EC 398889	Thailand
	For early maturity	IC 0589309, IC 0589310, IC 39289,	India
	Photo-insensitive	IC 0546478	India
	High seed weight	IC 0418452, IC 296771	India
	Pods per plant and seeds per pod	PLN 15	India
Urdbean	Photo-insensitive	INGR 13057, IC 426765	India
	Exceptionally large seed size; 100-seed weight 4.65 g	IC 573438	India
	Spontaneously occurring functionally male sterile mutant which produces flower with a protruded stigma and crumpled petals	IC 0594172	India
	The genotype has unique 'soybean' like sympodial pod bearing habit	IC 0594173	India
Mothbean (*Vigna aconitifolia*)	Drought tolerant	IC 296803	India
	Single stem-early maturity, high influx of sodium ions in root from soil	IC 432859	India

(continued)

Table 8.4 (continued)

Crop	Trait(s)	Accession(s)	Country of origin
	Extra early maturing (53 days)	IC 120963	India

Source Adopted and modified from Pratap et al. (2019) and database of ICAR-NBPGR Genebank

- Plant Gene Resources of Canada (http://pgrc3.agr.gc.ca/index_e.html)
- Ustymivka Experimental Station of Plant Production, Ukraine (https://www.genesys-pgr.org/wiews/UKR008)

Source Sivasankar et al. (2016).

8.4.2 Gene Pools of Mungbean and Urdbean

The gene pool concept was conceptualized by Harlen and De Wet (1971). It is very useful for plant breeders for initiating pre-breeding work for directed crop improvement (Kumar et al. 2011). Mungbean and urdbean genetic pool can be classified into primary, secondary, and tertiary gene pools (Table 8.2).

8.4.2.1 Primary Gene Pool (GP1)

The primary gene pool (GP1) is made up of members of the same species which can freely interbreed, resulting in viable/fertile offspring (hybrids). In crosses between members of GP1, normal chromosomal pairing and gene segregation can be seen, and generally gene transfer is easy. GP1 is of immense importance for breeding. The gene pool can be subdivided into two, subspecies A (cultivated races) and subspecies B (spontaneous races (wild/weedy).

8.4.2.2 Secondary Gene Pool (GP2)

The members of the secondary gene pool are considered as different species than the crop under consideration. These species are closely related to GP1 members and can cross with them to produce fertile/partially fertile hybrids. Crossing is possible, albeit challenging, to transfer genes from such material to the core gene pool. Reproductive barriers exist between members of the primary and secondary gene pool leading to production of partially sterile, weak hybrids. Pairing of chromosome is very poor or may be completely absent, resulting in difficulty in obtaining desired plant expression/phenotypes in next generations. Cytogenetical and biotechnological tools such as chromosome/ploidy level manipulation of parental or their hybrid derivatives, rescue of embryo through plant tissue culture techniques, use of bridge species,

Table 8.5 Sources of resistance for various abiotic stresses in mungbean/urdbean

Crop	Abiotic stress	Source of resistance/tolerance	References
Mungbean	Drought tolerance	V1281, V 2013, V3372	AVRDC (1979)
		VC1163D, VC2570A VC2754A, VC2768A	Fernandez and Shanmugasundaram (1988)
		TCR20	Tripathy et al. (2016)
		VC2917(seedling stage) and Zhang jiakouyinggelv (adult stage)	Wang et al. (2014, 2015a, b)
		K-851	Dutta and Bera (2008) Dutta et al. (2016) Kumar et al. (2020a, b)
		SML-1411, SML-1136	Kaur et al. (2017)
		ML267	Swathi et al. (2017)
		VRM (Gg)1 and VMGG012-005	Pandiyan et al. (2017)
		VC-6173-C, IC-325770, ML 2082	Raina et al. (2016)
		LGG 450, PUSA 9531, LGG 528, Asha, EC 396117 and MH 565	Paramesh et al. (2016)
		MH 810, MH 721, MH 736, M 395, SML 668, Pusa 9972	Sunayana et al. (2017)
		MGG 351	Govardhan et al. (2017)
		Vigna sublobata, MCV-1, PLM-32, LGG-407, LGG-450, TM-96-2, Sattya	Bangar et al. (2019)
		IPM 02-3-2, BIG-0068-1, MH421, EC 520014, IPM 9901-10, PDM 288	Manu et al. (2021)
	Drought and flood	V1381, VC2778A	He et al. (1988)
	Cold tolerance	OBGG-2013-8, OBGG-2013-9, OBGG-2013-34 and OBGG-2013-14	Kabi et al. (2017)
	High SPAD and better PSII health despite high canopy temperature	DMG-1050	Raina et al. (2019a, b)
	Drought and low phosphorus (P) tolerance	IC 280489, PDM 139, IC 76491	Meena et al. (2020)

(continued)

Table 8.5 (continued)

Crop	Abiotic stress	Source of resistance/tolerance	References
		IC 333090, IC 507340	Meena et al. (2021)
	Heat tolerance	EC 693357, EC 693358, EC 693369, Harsha and ML1299	Sharma et al. (2016)
		SML 1186, NDM 12-308, IPM 02-4, Smrat	Kumar et al. (2020a, b)
		EC 398889	Basu et al. (2019)
		IPM 06-5, IPM 02-3-2, BIG-0068-1	Manu et al. (2021)
	Water logging tolerance	V1968, V2984, V3092, V3372	AVRDC (1979)
		VC-6173A, BU mug 2 and IPSA-13	Amin et al. (2016)
		AVMU 1001, AVMU 1201, VO6381A-G, KPS-1, ML 1628, PDM 139, IPM 02-14	Rameshreddy et al. (2019)
		IPSA-10 and VC 6379 (23-11)	Shibly et al. (2020)
	Low temperature	Perennial accessions of *V. radiata* var. *sublobata*	Lawn et al. (1988)
	Salt tolerance	S72, H45, No. 525, Madira, RS-4	Maliwal and Paliwal (1982)
		TCR 86, PLM 380, PLM 562, WGG 37, IC 615, PLM 891	Sehrawat et al. (2014)
		EC 693357, 58, 66, 71 and ML 1299	Manasa et al. (2017)
	Alkaline and calcareous soil	Accessions of *V. radiata* var. *sublobata*	Lawn et al. (1988)
	Hardseededness (preharvest sprouting resistant)	*V. radiata* var. *sublobata*	Singh et al. (1983)
	Non shattering	Pant Moong-1	Singh and Sharma (1984)
	Pre-harvest sprouting tolerance	Chamu4	Lamichaney et al. (2017)
	Deep rooting	EC862594, IC 616203, IC 616109, IC616184 and EC 862589	Aski et al. (2021)
	Higher total surface area (root)	IC 616276	Aski et al. (2021)

(continued)

Table 8.5 (continued)

Crop	Abiotic stress	Source of resistance/tolerance	References
Urdbean	Drought tolerance	M-01001-1 and M-6036-21	Ali et al. (2016)
		VBN (Bg) 4 and VBN (Bg) 6	Pandiyan et al. (2017)
		CBG-09-13	Prakash et al. (2018)
		PGRU 95016, COBG 05, IPU 99209, IPU 941 and IPU 243	Gurumurthy et al. (2019)
		VBN4 and K1	Sai and Chidambaranathan (2019)
		VBG-12005, IC-343943 and IC-343947	Mohanlal et al. (2020)
	Cold tolerance	TCR-243 and TCR-20, B-3-8-8, OBGG-31, PU-35 and PU-30	Baisaki et al. (2014)
	Salt tolerance	LBG-738, LBG-648, LBG-708, LBG-723 and LBG-726	Shanti et al. (2014)
		VNBG 017, AUB 3 AND AUB 20	Priyadharshini et al. (2019)
		BARI Mash-1	Hasan et al. (2019)
	Flood tolerance	BU Acc 25, BU Acc 24, BU Acc 17	Rana et al. (2019a, b)
		IC 530491, IC 519330	Bansal et al. (2019)

production of alien addition and substitution lines and use of plant growth regulators (IAA, GA3, 2-4-D, etc.) for pre and post-fertilization barriers can be adopted to make crosses successful.

8.4.2.3 Tertiary Gene Pool (GP3)

Crossing members of the tertiary gene pool (GP3) with members of the primary gene pool (GP1) is extremely difficult and results in sterile hybrids. Members that can be crossed with GP1 are also included in GP3, however hybrids are infertile. GP1 members are distantly related to members of this group. It is extremely difficult to transfer genes from GP3 to GP1, and biotechnological technologies like as recombinant DNA technology, somatic cell hybridization, in-vitro fertilization, gene bombardment, and an agrobacterium-mediated approach can be fallowed. Interspecific hybridization research is critical for better understanding crop gene pools by

better understanding crossability relationships between species. This aids in the selection of F_1 hybrid production strategies as well as the tracing of evolutionary links between species in pre-breeding programmes. It also helps with biotic and abiotic stress resistance breeding, which improves yield and yield components. Introgressed materials resulting from extensive crosses may also serve as genetic reservoirs for novel genes that can act as novel donor lines. The *Vigna* gene pool has been evolving as a result of continued efforts to develop a crossable combination.

Various researchers have placed *V. aconitifolia* in the secondary gene pool (Pratap et al. 2014a) and Tomooka et al. (2011) in the tertiary gene pool, and few groups have placed *V. umbellata* in the secondary gene pool (Tomooka et al. 2011) and Pratap et al. (2014a) in the tertiary gene pool. Likewise *V. glabrescens* which is placed in the tertiary gene pool, isreported to give fertile progenies upon hybridization with the *V. radiata* without any pre/post syngamic barriers. Hence there is a need to revisit the *Vigna* gene pool based on hybridization studies and molecular maker tools.

8.5 Glimpses on Classical Genetics and Traditional Breeding

Mungbean and urdbean are deemed miracle crops, and plant breeders have been actively working to develop the crops. Susceptibility to various biotic/abiotic stresses and seed composition traits are two major issues with these crops. Improved crop agronomic output would result in increased productivity and efficiency, as well as increased mungbean and urdbean consumption and realized economic benefits. To choose better performing genotypes, plant breeders have traditionally used crossing approaches combined with careful selection methods. Many crop varieties have been developed using traditional plant breeding methods. Breeding activities for both crops are aimed at growing the crop's stable yield capacity. Drought tolerance, waterlogging stress, salt tolerance, and other abiotic stresses have all been developed using conventional genetics and breeding methods.

8.5.1 Classical Inheritance Studies

Classical genetics study in the mungbean began during the year 1930s. Several workers have made attempts to understand the genetics of quantitative/qualitative traits related to biotic/abiotic stresses.

Classical genetics studies involved study inheritance of several morphological traits such as leaf characters, plant type, pod pubescence, pod color, shattering habit, and seed coat color (Singh et al. 2017). Bose (1939) conducted the first genetic study to understand the inheritance of color of ripe pods and seed coat surface. A detailed review of the genetics of mungbean was done by Fery et al. (1980)

and Poehlman (1991). Lots of workers have studied inheritance pattern for biotic stresses but in abiotic stress one report is available, Nopparat et al. (1997) has studied the genetics of resistance to calcareous soil (Iron deficiency chlorosis), reported resistance is governed by 2 genes with inhibitory gene action. Srinives et al. (2010) reported, resistance to Iron deficiency chlorosis is controlled by a major gene (IR) with dominant effect along with modifying genes with minor effect conditioning the degree or level of the resistance.

In urdbean, many workers have reported inheritance patterns for morphological traits. Pathak (1961) reported hairy pods are dominant to non-hairy pods and controlled by a single gene. Black pod color is predominant in urdbean, other colours are brown and straw-coloured pods. Sen and jana (1964) reported black pod colour is dominant over brown and straw-coloured pods, governed by a single gene. Sen and Jana (1964) reported Shiny seed surface was dominant over the dull seed surface. Seed coat colour is another important trait, brown seed coat colour is recessive to green seed coat colour (Sen and Jana 1964). Another study by Arshad et al. (2005) reported that brown seed coat colour is dominant over green seed colour. First genetic linkage map of urdbean was constructed by Chaitieng et al. (2006) to compare with genetic linkage map of azuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi]. Genetic linkage map was constructed by using a BC_1F_1 population consisting of 180 individuals. The BC_1F_1 population was analysed in 61 SSR primer pairs, 56 RFLP probes, 27 AFLP loci and 1 morphological marker. Comparative genome mapping urdbean and azuki bean revealed, linkage order of markers is highly conserved. They also detected structural chromosomal aberrations such as deletions/duplications; insertions, inversions, and translocation were detected between the urdbean and azuki bean linkage maps.

Genetics, pattern of inheritance, and mode of gene action of various important traits of Mungbean and Urdbean are presented in Tables 8.6 and 8.7.

8.5.2 Classical Breeding Achievements

Development of Photo-thermo insensitive, short duration, resistance to pest and diseases were major breeding objectives during previous decades have helped in pushing these crops in newer ecological niches. Before, the mid-twentieth century breeders primarily sought for selecting superior cultivars from indigenous/exotic germplasms. Superior pure lines were selected based on progeny test, and released as varieties after testing for yield. The yield was the main focus at that time, once the pest and diseases caused more severe yield loss; the emphasis was given on breeding for biotic stress tolerance by identification of resistance source followed by their transfer into cultivated backgrounds by hybridization. Many workers have studied the genetics of important traits to include them in breeding programs to breed resistance to biotic and abiotic stresses. The main strategies employed by *Vigna* breeders were to breed high-yielding, stress resistant, short duration mungbean and Urdbean varieties by inter/intraspecific hybridization and mutation breeding. Breeding for

Table 8.6 Classical inheritance studies in mungbean

Trait	Inheritance and gene action	References
Plant type and growth habit	Single dominant/recessive gene, semi-spreading is dominant over erect habit	Pathak and Singh (1963) Khattak et al. (1999)
Pubescence	Single dominant gene	Murty and Patel (1973)
Nodulation	Additive and non-additive gene action	Singh et al. (1985)
Leaf traits	Single dominant gene, large leaflet is dominant over small leaflet; lobbed is dominant over entire type Unifoliate leaf is recessive to the normal trifoliate leaf controlled by a single gene	Singh and Singh (1995) Talukdar and Talukdar (2003) Jiao et al. (2019)
Flower color	Single dominant gene	Bose (1939)
Anthocyanin pigmentation (in peduncle, petiole, stem, hypocotyl lsland epicotyls reported Anthocyanin in hypocotyl	Single dominant gene Single recessive gene controlled by two supplementary genes viz. 'Sh' and 'Ph' with recessive epistatic interaction	Pathak and Singh (1963) Van Rheenen (1964) Virk and Verma (1977) Appa Rao and Jana (1973) Mukherjee and Pradhan (2002)
Pod color	Single dominant gene	Sen and Ghosh (1959) Murty and Patel (1973)
Pod shattering	Single dominant gene	Verma and Krishi (1969)
Seed coat color	One or few genes; mottling governed by single gene	Khattak et al. (1999), Chen and Liu (2001), Lambrides et al. (2004)
Seed coat surface	Two complementary genes	Sen and Ghosh (1959), Murty and Patel (1973)
Seed weight	Small seed size dominant over large	Fatokun et al. (1992). Humphry et al. (2005)
Hard seededness	One or few dominant genes involved	Lambrides (1996). Humphry et al. (2005)
Yield components	Additive and non-additive gene action	Dasgupta et al. (1998) Khattak et al. (2002)
Preharvest sprouting	Additive and non-additive gene action; high G × E interaction	Durga and Kumar (1997)

Source Adopted and modified fromPratap et al. (2019)

Photo thermo insensitivity, temperature stress tolerance, moisture, salinity stress, and pre-harvest sprouting needs more attention. Identification of sources from important economic traits, plant types, and desirable genes should be the major focus (Singh et al. 2016a, b). Classical breeding has given many high-yielding varieties (Tables 8.8 and 8.9) recommended different seasons and zones.

Table 8.7 Classical inheritance studies in urdbean

Trait	Inheritance	References
Erect plant type	Erect plant type is not completely dominant over spreading type	Sen and Jana (1964)
Dwarf (mutant)	Single recessive gene	Rao et al. (1975)
Ovate leaf shape	Single dominant gene, dominant over lanceolate leaf shape	Verma (1971)
Hastate leaf shape	Duplicate dominant gene, dominant over ovate leaf shape	Singh and Singh (1971)
Multifoliate leaves	Single recessive gene	Rao et al. (1989)
Fused leaf	Single recessive gene recessive to ovate leaf shape	
Hairy Pod	Single dominant gene, Hairy pods are dominant to non-hairy pods	Pathak (1961) Sirohi and Singh (1998)
Main stem bearing	Single dominant gene, incomplete in expression	Rao (1999)
Black pod colour	Single dominant gene, Straw pod colour and brown pod colour is recessive	Sen and Jana (1964), Verma (1971)
Small nonflowering bud or keel mutant	Single recessive gene	Appa Rao and Reddy (1976), Jana (1962)
Protruded stigma	Single recessive gene with pleotropic effects	Kumar et al. (2012)
Brown seed coat colour	Single recessive gene, recessive to green seed coat colour	Sen and Jana (1964)
Brown seed coat colour	Single recessive gene, recessive to green seed	Arshad et al. (2005)
Shiny seed surface	Shiny seed surface was dominant over dull seed surface	Sen and Jana (1964)

8.5.3 Limitations of Traditional Breeding and Rationale for Molecular Breeding

The varieties developed by traditional breeding methods are superior in one or more important traits, early maturity, plant type/improved grain yield. The superior varieties perform well under optimized climatic conditions but are susceptible when exposed to harsh climatic conditions; hence there is a need to make these crops climate-smart crops which will help in horizontal and vertical expansion of the mungbean and urdbean into newer ecological niches. Plant breeding in the twenty-first

Table 8.8 List of mungbean varieties released in India

S. No.	Name of variety	Year of release	Area of adaptation	Suitable for season
1	SML134	1996	Punjab	Spring/summer season
2	HUM1 (Malviya Jyoti)	1999	CZ and SZ	*Kharif* season
3	RMG268	1997	Rajasthan	*Kharif* and summer season
4	CO 6	1999	TN	Suitable for all seasons
5	HUM2 (Malviya Jagrati)	2000	UP and Uttarakhand	Spring/summer season
6	PDM139	2001	UP and plains of Uttrakhand	Spring/summer
7	PantMung5	2002	UP and plains of Uttrakhand	–
8	IPM 02-3	2009	NWPZ	*Kharif* and spring season
9	PKVAKM4	2009	CZ and SZ	*Kharif* season
10	MH421	2014	NWPZ	Summer/spring
11	Yadadri (WGG 42)	2016	Telangana	*Kharif*/*Rabi* and summer cultivation
12	IPM205-7 (Virat)	2016	Entire India	Summer
13	IPM410-3 (Shikha)	2016	NWPZ/CZ	Summer/spring
14	Sri Rama (MGG351)	2016	Telangana	*Rabi*/summer and rice fallow
15	MSJ118 (Keshvanand mung)	2016	Rajasthan	*Kharif*/spring cultivation
16	GAM5	2018	Gujarat	Summer and *Kharif* season
17	SGC16 (Rupohi)	2018	Assam	Summer and season
18	IPM 512-1 (Soorya)	2020	NEPZ	Suitable for spring season
19	MH 1142	2020	NEPZ and NWPZ	Suitable for *kharif* season

Source Project Coordinator's Report, AICRP on MULLaRP, ICAR, IIPR, Kanpur 2020–2021

century is a combination of the genome, germplasm phenotyping, and data science, this combination is essential to make crops climate-smart. Major abiotic stresses, like drought, waterlogging salinity, extreme temperature are complex in nature, governed by polygenes producing a range of phenotypes. Studying these traits requires destructive sampling, collected from stress-exposed environments. Phenotype is growth stage-dependent and exposure to stress environments makes it difficult to recover valuable germplasm material. Traditional breeding is time-consuming, laborious and

Table 8.9 List of urdbean varieties released in India

S. No.	Name of variety	Year of release	Area of adoption	Suitable for season
1	Himachal Mash1	2007	Lowhills of Himachal Pradesh	
2	DU-1	2008		*Kharif*/*rabi*/summer/paddy fallows
3	Mash114	2008	Punjab	
4	LBG752	2009	Andhra Pradesh	
5	CO6/COBG653	2009	TN, AP, Orissa	
6	VBN6	2011	Tamilnadu	
7	UH-1	2012	Haryana	Irrigated
8	DBGV-5	2014	Karnataka	*Kharif*
9	Pratap Urd-1 (KPU07-08)	2013	Rajasthan	
10	SBC40	2014	Assam	
11	MDUI	2014	Tamilnadu	
12	Vallabh Urd1	2015	Uttar Pradesh	
13	Indira Urd Pratham	2016	Chhattisgarh	*Kharif* and summer
14	Tirupati Minumu-1 (TBG104)	2016	Andhra Pradesh	*Rabi*
15	PDKV Blackgold (AKU10-1)	2016	Maharashtra	*Kharif*
16	ADT6	2017	Tamilnadu	Rice fallow
17	KKM-1	2017	Tamilnadu	*Kharif* and Rice fallow
18	Pant Urd 10	2019	NHZ	Suitable for *kharif* season
19	VBN 9	2020	SZ	Suitable for rice fallow cultivation
20	VBN 10	2020	SZ	Suitable for *rabi* cultivation

Source Project Coordinator's Report, AICRP on MULLaRP, ICAR, IIPR, Kanpur 2020–2021

breeders face linkage drag during hybridization program requiring several generations of selection and backcrossing rounds before presenting the cultivar for release (Flint-Garcia et al. 2003). Molecular tools are plant growth stage independent for screening purposes helps to save time, fastens decision making, thereby saving cost involved in the cultivation of large population. Molecular breeding involves the use of molecular markers in mapping genes/QTLs. Since selection is growth stage independent it aids in the precise selection of desirable alleles increasing the selection intensity. Molecular breeding leads to the development of genomic resources for a better understanding of the genomic structure, accelerates breeding efforts and genetic techniques such as marker-assisted breeding, recombinant DNA technology, genome editing, and "omics" could be used to boost the quality and yield of mungbean

and urdbean varieties. Marker trait associations will help in the precise manipulation of target genes for a key agronomic trait which can help in the development of tolerance against different abiotic stresses (Singh et al. 2016). Identification of unique alleles from wild species helps in diversity studies and re-domestication.

8.6 Diversity Analysis

8.6.1 Phenotype-Based Diversity Analysis

Phenotyping-based diversity analysis involved study of morphological traits which are highly inheritable and qualitative in nature. Phenotypic based diversity study is the first and foremost important activity for the utilization of germplasm in crop improvement programs. Recording of plant descriptors in optimum or adverse environmental conditions to reveal its potential useful variability available within the germplasm may be termed as evaluation. These traits are quantitative in nature and are of immense importance for crop improvement programmes, breeding for yield and yield attributing traits. Phenotype-based diversity analysis helps in identifying trait-specific germplasm. Kawalkar et al. (1996) studied 1532 accessions of mungbean using 19 qualitative and 19 quantitative traits. Schafleitner et al. (2015) characterized global mungbean accessions ($n = 5234$) for eight agro-morphological traits and reported good amount of phenotypic variability with Shanon's diversity index which was 0.82 (average of all traits). Several *Vigna* scientists have studied diversity in mungbean and urdbean germplasm to understand genetic variability, genetic divergence, and trait association (Bisht et al. 1998b; Chattopadhyay et al. 2008; Yimram et al. 2009; Tantasawat et al. 2010; Rahim et al. 2010; Abna et al. 2012; Singh et al. 2014; Hakim 2016). Using Metroglyph analysis and a variety of morphological and economic characteristics, the genetic variation of mungbean germplasm was investigated (Abbas et al. 2010). Morphological traits and RAPD profiles were used to determine the extent of diversity among 54 mung bean accessions, which included both improved and local land races (Lavanya et al. 2008). A study was carried out on mungbean accessions using Relative high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) among 8 quantitative, 18 qualitative traits, and four seed morps (Gayacharan et al. 2020).

8.6.2 Molecular Marker Assisted Assessment

Study of genetic diversity of a crop is of utmost importance to know the extent of genetic variability available in the germplasm. It helps in understanding genetic relationship among the germplasm groups, helps in study of population genetics, evolutionary relations, taxonomy and phylogeny of germplasm available. Use of molecular markers results in accurate estimation of genetic diversity. Because, molecular markers unlike morphological markers are stage independent and are not influenced by environment. Genetic markers provide information regarding on homologous loci among genotypes, while morphological traits may be governed by multiple genes, making it difficult to study allelic relationships.

Morphological markers are limited in numbers while molecular markers are abundance in number resulting in increasing the power to discriminate between genotypes besides being easily score able. This helps in precise estimation of genetic diversity leading toselection of diverse parental combinations hybridization programme leading to directed accumulation of favourable alleles. Use of molecular markers in diversity studies, removes the duplicity and misidentify in the core accessions. Several marker technologies have been used to characterize mungbean germplasm. Use of restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), and inter-simple sequence repeats (ISSR) and SSR (Simple sequence repeats) markers have dominated the literature on genetic diversity studies in mungbean and urdbean over the last decades. List of marker system used in mungbean and urdbean for genetic mapping/diversity studies has been given in Table 8.10.

Table 8.10 List of marker system used in mungbean and urdbean for genetic mapping/diversity studies

S. No.	Maker name	References
1	RFLP	Fatokun et al. (1992), Young et al. (1992, 1993), Menancio-Hautea et al. (1993), Selvi et al. (2006), Chen et al. (2007)
2	RAPD	Santalla et al. (1998), Lakhanpaul et al. (2000), Datta et al. (2012)
3	RAPD and ISSR	Chattopadhyay et al. (2005)
4	AFLP	Bhat et al. (2005), Singh et al. (2013)
5	SSR	Sangiri et al. (2008), Gwag et al. (2010), Isemura et al. (2012), Gupta et al. (2014), Kaewwongwal et al. (2015), Chen et al. (2015a)
6	EST-SSR	Chen et al. (2015b)
7	Morphological and SSR	Schafleitner et al. (2015) (geographic stratification)
8	SNP	Moe et al. (2011), Van et al. (2013), Kang et al. (2014), Liu et al. (2016), Schafleitner et al. (2016), Islam and Blair (2018), Noble et al. (2018), Breria et al. (2019)

8.6.3 Relationship with Other Cultivated Species and Wild Relatives

Vigna is a large genus consisting of cultivated crops and wild relatives found throughout Asia and Africa. At present, genus *Vigna* is divided into five subgenera: *Ceratotropis*, Haydonia, Lasiospron, *Plectrotropis*, and *Vigna*. Out of which three of the subgenera include crop species: *Plectrotropis* and *Vigna*, which originated in Africa, include cowpea, Bambara nut (*Vigna subterranea* (L.) Verdc.) and tuber cowpea (*Vigna vexillata* (L.) A. Rich.), and *Ceratotropis*, which originated in Asia, and also known as the Asian *Vigna*, which is agronomically most important taxonomic group, having seven domesticated crops, i.e., moth bean (*Vigna aconitifolia* (Jacq.) Maréchal), minni payaru (*Vigna stipulacea* Kuntze), mung bean (*Vigna radiata* (L.) R. Wilkzek), urdbean (*Vigna mungo* (L.) Hepper), creole bean (*Vigna reflexo-pilosa* Hayata), rice bean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi), and adzuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi).

According to Verdcourt (1970), Tateishi and Ohashi (1990), Maxted et al. (2004), the subgenus *Ceratotropis*is believed to have emerged from the subgenus *Vigna* via the subgenus *Plectrotropis*. However, study by Takahashi et al. (2016) on the DNA sequences of nuclear rDNA-ITS and chloroplast atpB-rbcL spacer regions, suggested common ancestor of the genus *Vigna* first diverged into the common ancestor of the subgenera *Vigna* plus *Plectrotropis*, and the common ancestor of the subgenus *Ceratotropis*. Then, the common ancestor of the subgenera *Vigna* plus *Plectrotropis* diverged into the common ancestor of the section *Vigna* (subgenus *Vigna*) and the common ancestor of the section *Catiang* (subgenus *Vigna*) plus subgenus *Plectrotropis*. Under subgenus *Ceratotropis*, Tomooka et al. (2002a) Listed 21 species and established three sections: Aconitifoliae, *Ceratotropis*, and Angulares. As suggested by Takahashi and Tomooka (2020) in the last 10 years there has been a discovery of six new species in subgenus *Ceratotropis* of genus *Vigna*, signifying the importance of systematic germplasm exploration should be of high priority since some of the wild habitat might be lost in near furure. Value of the germplasm resources can be enhanced greatly by carefully observing discriptors at field level the ecological adaptations of wild plants to their specific habitats. These studies will regularly update and expand gene pool of related crop species. Diagnostic characters for distinguishing three sections and their distribution in the subgenus *Ceratotropis* has been given in Table 8.11.

8.6.4 Relationship with Geographical Distribution

The genus *Vigna* consists of 104 species distributed all over tropical and subtropical regions of Asia, America, Africa, and Australia (Lewis et al. 2005) (Table 8.12). It is important and interesting leguminous taxon consisting up to nine domesticated

Table 8.11 Diagnostic characters for distinguishing three sections and their distribution in the subgenus *Ceratotropis*

Characters	Sections under subgenus *Ceratotropis*		
	Aconitifoliae	*Ceratotropis*	Angulares
Germination	Epigeal	Epigeal	Hypogeal
Appendages on standard	Absent	Present	Present
Primary leaves	Both	Sessile	Petiolate
Species	*Vigna aconitifolia* (Jacq.) Marechal *Vigna aridicola* N. Tomooka et Maxted *Vigna indica* T. M. Dixit, K V. Bhat et S. R. Yadav *Vigna khandalensis* (Santapau) Raghavan et Wadhwa *Vigna stipulacea* (Lam.) Kuntze *Vigna subramaniana* (Babu ex Raizada) Raizada *Vigna trilobata* (L.) Verdc	*Vigna grandiflora* (Prain) Tateishi et Maxted *Vigna konkanensis* Latha, K. V. Bhat, I. S. Bisht, Scariah, Joseph John et Krishnaraj *Vigna mungo* (L.) Hepper *Vigna pandeyana* R. D. Gore, S. P. Gaikwad et S. D. Randive *Vigna radiata* (L.) Wilczek *Vigna sahyadriana* Aitawade, K. V. Bhat et S. R. Yadav *Vigna sathishiana* A. P. Balan et S. V. Predeep	*V. angularis* (Willd.) Ohwi & Ohashi *Vigna dalzelliana* (Kuntze) Verdc *Vigna exilis* Tateishi et Maxted *Vigna hirtella* Ridley *Vigna minima* (Roxb.) Ohwi et Ohashi *Vigna nakashimae* (Ohwi) Ohwi et H. Ohashi *Vigna nepalensis*Tateishi et Maxted *Vigna reflexo-pilosa*Hayata *Vigna riukiuensis* (Ohwi) Ohwi et Ohashi *Vigna tenuicaulis* N. Tomooka et Maxted *Vigna trinervia* (Heyne ex Wight et Arn.) Tateishi et Maxted *Vigna umbellata* (Thunb.) Ohwi et Ohashi *Vigna yadavii* S. P. Gaikwad, R. D. Gore, S. D. Randive et K. U. Garad
Distribution	South Asia	South Asia to Southeast Asia	Southeast Asia to East Asia

Source Adopted and modified from Takahashi and Tomooka (2020)

species which are grown as food crops in three different continents Asia, America and Africa (Table 8.13).

Mungbean is believed to be originated in Indian gene center (Jain and Mehra 1980) and it's a native of indo-Burma regions of Asia where it was first domesticated by its wild progenitor designated as *V. radiata* var. *sublobata* and are of Indian origin (Chandel et al. 1984), distributed in Himalayan foothills and Tarai regions, and sporadically in western and eastern peninsular tracts of India (Arora and Nayar 1984). Urdbean is considered to be domesticated in India from its wild progenitor,

Table 8.12 Gene pool classification of genus *Vigna* by post-hoc tukey honest significant difference (HSD) tests

Gene pool	Subgenus	Species
A	*Ceratotropis*	*V. radiata* (mung bean), *V. mungo* (urd bean), *V. angularis* (azuki bean), *V. umbellata* (rice bean), *V. reflexo-pilosa* var. *glabra* (creole bean), and *V. aconitifolia*
B	*Plectotropis*	*V. vexillata*
C	Sections *Catiang*, *Macrodontae*, and *Reticulatae* of subgenus *Vigna*	*V. unguiculata* group *sesquipedalis, V. unguiculata* group *unguiculata*
D	Section *Vigna*of subgenus *Vigna*	*V. subterranean, V. marina* and *V. luteola*

Source van Zonneveld et al. (2020)

(*Vigna mungo*var. *silvestris*) Lukoki, Maréchal, and Otoul (Chandel et al. 1984), distributed in India, Bangladesh, Pakistan, West Myanmar (Tateishi 1996). Mungbean has spread to many countries, especially in tropical and subtropical Asia, the Urdbean has remained more or less confined to South Asia (Nene 2006). India is the primary centre of diversity for mungbean (Arora 1988) and urdbean domesticated in northern South Asia from *V. mungo* var. *silvestris* that commonly grows there (Luloki et al. 1980; Fuller 2002). Secondary center of diversity for mungbean being the Indo-gangetic plains (Bisht et al. 1998a). Mungbean and Urdbean finds mention in the historical texts, Urd in *Kautilya's Arthasasthra*' and '*Charak Samhita*' and mung in *Yajurveda* (c. 7000 BC). Sanskrit name for mungbean has been '*mudga*' and '*masha*' *for* urdbean. The name urad is used, which seems to have originated from the Tamil word '*ulundu*' (Nene 2006). India is the only country where archaeological seed remains of urdbean has been found with oldest seeds found maybe dating back about 4500 to 5500 years BP (Fuller and Harvey 2006). Mungbean can be considered as one most widely distributed among the Asiatic *Vigna* species. Presently it is cultivated throughout the South and Southeast Asia, which includes countries like India, Bangladesh, Myanmar, Sri Lanka, Pakistan, Indonesia, Malaysia, Thailand, Vietnam, Philippines, Laos, Cambodia, South China, and Taiwan. Mungbean was grown in USA as Chickasaw pea as early as 1835. In Africa and Australia also it is grown in lesser extent however it has not become a major commercial crop in these countries. In recent times Urdbean is introduced to Africa and America by Indian immigrants (Jain and Mehra 1980) where it is grown as food and manure crop, and is called "*woolly pyrol*" in America. Mungbean too was carried by emigrants and traders from Asia to the Middle East, East Africa, Latin America, parts of South America and Australia (Poehlman 1991). Wild urdbean and wild mungbean populations In India, generally have the similar geographical distribution, although some show distinct distribution (Bisht et al. 2005). In India Mungbean is mainly grown in states of Odisha, Andhra Pradesh, Maharashtra, Rajasthan, Bihar, Gujarat, and Madhya Pradesh and Urd in states of Andhra Pradesh, Punjab, Tamilnadu, Orissa,

Table 8.13 Geographical distribution of distribution of germplasm of *Vigna* species and its genomic resources

Species name	Common name	Chromosome number	Origin areas	Cultivation	Genome sequence availability	References
Vigna radiata	Mungbean	$2n = 2x = 22$	South Asia	South, East, and Southeast Asia	Available	Nair et al. (2012) Kang et al. (2014)
Vigna mungo	Urdbean	$2n = 2x = 22$	South Asia	South and Southeast Asia	Available	Gupta et al. (2013) Kang et al. (2015)
Vigna aconitifolia	Moth bean	$2n = 2x = 22$	South Asia	India and the Far East	Not available	Adsule (1996)
Vigna angularis	Adzuki bean	$2n = 2x = 22$	East Asia	China, Japan, Korean peninsula	Available	Kaga et al. (2008) Kang et al. (2015)
Vigna reflexo-pilosa	Creolebean	$2n = 2x = 44$ (Tetraploid)	South east Asia	Vietnam, Philippines (as pulse); India, Mauritius, and Tanzania (as forage)	Not available	Tomooka et al. (2002b)
Vigna trilobata	Junglebean	$2n = 2x = 22$	South Asia	Africa, Australia, Madagascar, Mauritius, and South America	Not available	Kaur and Kishore (2012)
Vigna trinervia	Tooapee (Thai)	$2n = 2x = 22$	South and Southeast Asia	Madagascar, South India, Sri Lanka, Myanmar, Malaysia, Sumatra, Java, Timor, and New Guinea	Not available	Tateishi (1985)

(continued)

Table 8.13 (continued)

Species name	Common name	Chromosome number	Origin areas	Cultivation	Genome sequence availability	References
Vigna umbellata	Rice bean	$2n = 2x = 22$	Southeast Asia	Fiji, Australia, tropical Africa, Indian Ocean Islands, USA, Honduras, Brazil, and Mexico	Not available	Khadka and Acharya (2009)

Source Adopted from Shanthala et al. (2020)

Haryana, Karnataka and Rajasthan. In general *Vigna* species and wild relative's thrives well in hot humid weather of subtropical to tropical regions.

To provide insights in patterns of distribution of abiotic and biotic stress resilience across *Vigna* gene pools and to through light on conservation and use of genetic resources for legume breeding. van Zonneveld et al. (2020) performed the ecogeographic analysis to identify *Vigna* species that occur in harsh climatic conditions and proposed 4 *Vigna* gene pools (Table 8.12) namely Gene pool A, Gene pool B, Gene pool C and Gene pool D. They reported that less than 30% of the taxa have resilience abiotic stress, and revealed that during evolutionary process *Vigna* taxa tend to easily acquiring, phenological traits for short life cycles, to escape drought and heat stresses compared with acquiring physiological traits to tolerate these stresses continuously. They also reported that *Vigna* taxa are good at developing salt-tolerant traits compared with drought-tolerant traits, as they found salinity tolerance in 27 percent of taxa compared with eight percent drought-tolerant taxa.

8.7 Molecular Mapping Tolerance Genes and QTLs

8.7.1 Molecular Marker Development

DNA based marker systems such as restriction fragment length polymorphisms (RFLPs), Random amplification of polymorphic DNA (RAPD), Simple sequence repeats (SSRs) or microsatellites, Amplified fragment length polymorphism (AFLP), single-nucleotide polymorphism (SNP) and Diversity arrays technology (DArT) have been used in several molecular breeding experiments. Among these marker systems, RAPD, RFLP and AFLP are commonly employed for marker trait association and diversity analysis in pulses, but not for MAS because of their poor reproducibility,

need of skilled man power, handling difficulties, and use of radioactive elements for generating these markers. Only PCR based markers like SSR and SNP have been preferred because of their ease in use, cost effective nature and high reproducibility. First report of using RFLP marker in mungbean for mapping traits was published by (Fatokun et al. 1992; Young et al. 1992). Menancio-Hautea et al. (1993) constructed genetic map based on RFLPs consisting of 171 loci on 14 linkage group. Many workers while studying genetic diversity have resorted to use of RAPD marker system. Santalla et al. (1998) have used RAPD in studying genetic diversity in germplasm and in cultivars (Lakhanpaul et al. 2000). Selvi et al. (2006) has used RAPD marker system to map resistance to mungbean yellow mosaic disease and bruchid beetles by Chen et al. (2007). Since RAPD is not reproducible *Vigna* scientists moved towards more reproducible marker system to improve genetic studies. Bhat et al. (2005) and Singh et al. (2013) used AFLP marker to study genetic diversity in mungbean, and for trait mapping (Chaitieng et al. 2006; Srinives et al. 2010). However the developed marker system wear not used by plant breeding due to requirement of skilled man power, handling difficulties, and use of radioactive elements. With the generation of sequence information in various *Vigna* species large number of SSR markers were assembled for mungbean (Somta et al. 2009) or were generated for mungbean genomic sequences (Tangphatsornruang et al. 2009) or transcriptome sequencing data (Gupta et al. 2014; Chen et al. 2015a). SSR makers were used for constructing genetic map (Kajonphol et al. 2017), domestication-related traits (Isemura et al. 2012) and nutritional traits such as phytic acid content (Sompong et al. 2012), to study diversity (Sangiri et al. 2008; Gwag et al. 2010; Chen et al. 2015a) and establish mini core collection (Schafleitner et al. 2015). Many workers have tried to generate sequence information on simple sequence repeats and single nucleotide polymorphisms with the help of transcriptome sequencing, in mungbean (Moe et al. 2011.) and in Urdbean (Jasrotia et al. 2017; Raizada and Souframanien 2019; Souframanien and Reddy 2015).

Van et al. (2013) by comparing the reads obtained by Illumina HiSeq sequencing of the genomes two mungbean cultivars. With the availability of whole genome sequence of mungbean cultivar VC1973A (Kang et al. 2014) and in urdbean Souframanien et al. (2020) constructed a draft genome sequence of urdbean, for the first time, by employing hybrid genome assembly with Illumina reads and third generation Oxford Nanopore sequencing technology. The whole genome assemblies support genome-wide association study (GWAS) studies to identify trait-specific loci and for genomic based selective breeding. With the availability of whole genome sequence of mungbean cultivar VC1973A (Kang et al. 2014), further developments on genotyping by sequencing approaches were undertaken (Kang et al. 2014; Schafleitner et al. 2016). Current re-sequencing projects producing huge numbers of markers are likely to provide insight into genome re-arrangements in these crops.

8.7.2 *Quantitative Trait Locus (QTL) Mapping for Abiotic Stress Tolerance*

Several attempts have been made to map the key quantitative trait loci (QTLs, Table 8.14) controlling economically important traits using different types of DNA markers through both conventional linkage and association mapping approaches. The availability of the entire genome sequence and affordable high performance genotyping tools, such as genotyping by sequencing, facilitates mapping of breeder desired traits.

Drought is one of the most serious constraints hampering *Vigna* production. Since abiotic stresses are complex in nature it's very difficult to understand genetic mechanisms controlling tolerance/susceptibility to abiotic stresses. Isolation of drought-responsive genetic elements and marker-assisted selection breeding will help in genomic-assisted breeding for abiotic stress-tolerant varieties.

Liu et al. (2017) mapped QTLs for drought tolerance traits using a recombinant inbred line (RIL) population derived from an intra-specific cross between two drought-resistant varieties. A novel genetic linkage map anchored with 313 markers was constructed by using SSR markers covering all linkage groups. Eleven linkage groups had a total map length of 1010.18 cm covering the entire genome of mungbean with a saturation of one marker every 3.23 cm. QTLs were mapped for 6 drought tolerance related-traits using single-environment analysis under irrigation and drought treatments. Fifty-eight QTLs for plant height (PH), maximum leaf area (MLA), biomass (BM), relative water content, days to first flowering, and seed yield (Yield) were reported. Out of which 38 QTLs were consistently detected two or more times at similar linkage positions. QTLs loci reported were never previously identified. Muchero et al. (2009) reported the mapping of 12 quantitative trait loci (QTL) associated with seedling drought tolerance and maturity in cowpea recombinant inbred (RIL) population (127 lines) developed from a cross between IT93K503-1 and CB46 and screened with 62 EcoR1 and Mse1 primer combinations to generate 306 amplified fragment length polymorphisms for use in genetic linkage and QTL mapping. Observed QTL were highly reproducible. Regions harboring drought-related QTL were observed on linkage groups 1, 2, 3, 5, 6, 7, 9, and 10 accounting for between 4.7 and 24.2% of the phenotypic variance (R2). Further, two QTL for maturity (R2 = 14.4–28.9% and R2 = 11.7–25.2%) mapped on linkage groups 7 and 8 separately. Muchero et al. (2010) again validated the consistency of the QTL by AFLP markers and found that few of their QTL identified previously (Muchero et al. 2009) were consistent. Sai and Chidambarnathan (2019) showed two varieties namely VBN4 and K1 have higher tolerance due to increased synthesis of ABA (fivefold), proline (4.5-fold), and lipid peroxidase activity (fivefold) which collectively protects tissues from oxidative damage during drought stress. However, their SDA-PAGE and mRNA expression analysis during drought indicated differential expression at 23 KDa molecular weight and with 1100 bp region respectively in the drought-stressed *Vigna mungo* VBN4 samples homological to chloroplastic small HSPs.

Table 8.14 List of QTLs mapped in *Vigna* spp. for various traits affecting abiotic stress

Crop/wild spp.	S. No.	QTL	Trait(s)	Location/linkage group	Marker	Mapping population	References
Mungbean (*Vigna radiata*)	1	4 QTLs, 11 QTLs	Hard seededness, seed weight	–	RFLP	RIL	Humphry et al. (2005)
	2	1 QTL	Iron deficiency chlorosis (IDC)	*qIR* controlling resistance to IDC located at 26.4 cM of a partial linkage map which explains (76.39%) of PVE	AFLP	F2	Srinives et al. (2010)
	3	20 QTL	Days to 1st flower	4 QTLs, LG-2 (15.88%), LG-4 (7.39%), LG-4 (28.57%), LG-11 (6.28)	SSR	F2	Kajonphol et al. (2017)
			Days to first podmaturity	3 QTLs, LG-2 (12.58%), LG-4 (8.43%), LG-4 (27.83%)			
			Daysto harvest	3 QTLs, LG-2 (15.60%), LG-4 (11.73%), LG-4 (11.63%)			

(continued)

Table 8.14 (continued)

Crop/wild spp.	S. No.	QTL	Trait(s)	Location/linkage group	Marker	Mapping population	References
			100 seedweight	6 QTLs, LG-2 (14.56%), LG-2 (12.99%), LG-4 (11.96%), LG-8 (8.16%), LG-9 (7.22%), LG-11 (8.74%)			
			No. of seed/pod	2 QTLs, LG-1 (12.29%), LG-1 (12.08%)			
			Podlength	2 QTLs, LG-7 (10.74%), LG-8 (9.26%)			
	4	3 QTLs	Iron deficiency chlorosis (IDC)	2 QTLs, qIDC3.1 (12.12%) and qIDC2.1 (41.67%) on LG 3 1 QTL, qIDC2.1 (45.66%) on LG 2	AFLP, SSR	RIL	Prathet et al. (2012)
	5	46 QTLs	Seed permeability	(4 QTL), LG-1, LG-2, LG-3, LG-4 (33.7%),	SSR		Isemura et al. (2012)
			Pod dehiscence	(2 QTL), LG-1, LG-7 (10.8–12.7%)			
			PDRW	(3 QTL), LG-1 (20%), LG-6, LG-7			

(continued)

Table 8.14 (continued)

Crop/wild spp.	S. No.	QTL	Trait(s)	Location/linkage group	Marker	Mapping population	References
			Seed size related traits	(5–7 QTL), LG-8 (15.1–22.7%), LG-2 (11.4–16.6)			
			Pod length (5 QTL), Pod width (4 QTL)	LG-2 (20.5%), LG-7, LG-8 (28.5%)			
			Primary leaf width (1 QTL)				
			Stem thickness (1 QTL)	LG-2 (10.2%)			
			Branch number (3 QTL)	LG-2 (22.3%), LG-4, LG-6			
			Flowering time (4 QTL)	LG-2 (32.9%), LG-4, LG-6, LG-11			
			Days to pod maturity (6 QTL)	LG-2 (20.3%), LG-4 (19.9%), LG-6, LG-7, LG-9, LG-11			
			Total number of pod	(4 QTL) LG-2, LG-4, LG-7 (5.8–12%)			
			Seed number/pod	(2 QTL) LG-1 (7%), LG-2 (9.1%)			
	6	4 QTLs,	Seed weight	LG-1 (5.80%), LG-6 (19.96%), LG-8 (8.31%) and LG-9 (33.72%)	SSR	$F_{2:3}$	Alam et al. (2014)

(continued)

Table 8.14 (continued)

Crop/wild spp.	S. No.	QTL	Trait(s)	Location/linkage group	Marker	Mapping population	References
	7	58 QTLs	Plant height	16 QTLs (2.86–20.06%)	SSR	RIL	Liu et al. (2017)
			Maximum leaf area (MLA)	12 QTLs (3.49–20.59%)			
			Biomass	12 QTLs (3.19–29.45%)			
			Relative water content (RWC)	6 QTLs (6.64–14.59%) on LG04, LG08, and LG10			
			Days to first flowering (FLD)	8 QTLs on LG04, (8.71–28.38%)			
			Seed yield (Yield)	4 QTLs on LG01, LG04, and LG08			
			Drought tolerance index	2 QTLs (qPHI4.1 and qPHI4.2) (7.85 and 21.60%)			

(continued)

Table 8.14 (continued)

Crop/wild spp.	S. No.	QTL	Trait(s)	Location/linkage group	Marker	Mapping population	References
Urdbean (*Vigna mungo*)	8	42 QTLs	To determine the effect of the *mog* gene (multiple-organ gigantism (MOG)) on agronomic and adaptive traits, yield-related and plant architectural traits	42 QTLs PVE (2.73–40.21%)	SNP	RIL	Somta et al. (2020)
Beach cowpea (*Vigna marina*)	9	11 QTLs	Pod length	(3 QTL) LG-3 (13.84%), LG-8 (8.81%), LG-9 (6.76%)	SSR	F2	Chankaew et al. (2014)
			100-seed weight	(4 QTLs) LG-2 (6.15%), LG-4 (20.6%), LG-9 (22.7%), LG-10 (7.69%)			
			Percentage of surviving seedlings	(1QTL) LG-1 (50.7%)			
			Leaf wilt score at vegetative stage	(1QTL) LG-1 (41.4%)			
			Plant recovery score	(1QTL) LG-1 (20%)			

(continued)

Table 8.14 (continued)

Crop/wild spp.	S. No.	QTL	Trait(s)	Location/linkage group	Marker	Mapping population	References
Yardlong bean (*Vigna unguiculata* (L.) Walp. subsp. unguiculata Sesquipedalis Group)	10	7 QTLs	Pod length	(7 QTLs 80.5%) PVE, LG01, LG03, LG04, LG05, LG07, LG08, and LG11	SSR	BC1F1 and F2	Kongjaimun et al. (2012)
Zombi pea (*Vigna vexillata*)	11	37 QTLs	18 domestication-related traits	37 QTLs (6.37–52.16%) PVE	SSR	F2	Dachapak et al. (2018)
	12	62 QTLs	13 domestication-related traits	62 QTLs (1.39–37.72%) PVE	SNP	F2	Amkul et al. (2020)

QTL mapping for salinity tolerance has been studied in several legume species, indicating that this trait is controlled by multiple (both major and minor) QTLs, like a single QTL (R_2) contributed roughly 9.5–12.5% of the phenotypic variation in Medicago truncatula (Arraouadi et al. 2012); a major QTL accounted for 44.0 and 47.1% of the total variation in salt tolerance for two populations of soybean (*Glycine max* (L.) Merr.) (Hamwieh et al. 2011); two QTLs, respectively, explained 12% and 19% of the phenotypic variance in salt index scores in field pea (*Pisum sativum* L.) (Leonforte et al. 2013); and two major QTLs associated with yield during salt stress explained 12 and 17% of the phenotypic variation in chickpea (*Cicer arietinum* L.) (Pushpavalli et al. 2015).

Very few studies have been conducted to map salinity tolerance in *Vigna* species like a major QTL which explained ~50% of the salt tolerance-related phenotypic variance in beach cowpea [*Vigna marina* (Burm.) Merrill] has been reported (Chankaew et al. 2014). Similarly, Zhang et al. (2020) identified six major QTLs associated with control of salt tolerance related traits on chromosomes 08, 09, and 11, which explained 9.9–72.7% of the phenotypic variation. Chankaew et al. (2014) performed QTL mapping in an F2 population of 120 plants using 150 markers derived from *V. luteola* × *V. marina* subsp. *Oblonga* and found that salt tolerance in *V. marina* subsp. *oblonga* is controlled by a single major QTL explaining more than 50% of the phenotypic variance. Similarly, a zombi pea F2 population (159 individuals) developed from a cross between the salt-resistant wild zombi pea accession JP235908 (var. ovata; female parent) and the salt susceptible cultivated zombi pea accession TVNu240 (var. macrosperma; male parent) was genotyped with simple sequence repeat (SSR) and restriction site-associated DNA sequencing (RAD-seq) markers followed by composite interval mapping (Dachapak et al. 2019). QTL mapping identified three quantitative trait loci (QTLs) (*qSaltol1.1* on LG1, *qSaltol2.1* on LG2, and *qSaltol6.1* on LG6) related to the salt resistance explaining 13.3, 7.6, and 8.1% of the phenotypic variance, respectively. Comparative genome analysis revealed 1 QTL conferring salt tolerance in beach cowpea [*Vigna marina* (Burm.) Merr.] That is *qSaltol1.1* may correspond to the *Saltol1,* in beach cowpea; beach cowpea is a halophytic species.

Ravelombola et al. (2018) studied the salt tolerance index of cowpea accessions at germination ($n = 116$) and seedling stages ($n = 155$). For association analysis, a total of 1049 SNPs were postulated from genotyping-by-sequencing. They reported, three SNPs, Scaffold 87490_622, Scaffold 87490_630, and C35017374_128 were highly associated with salt tolerance at the germination stage. At seedling stage seven SNPs, Scaffold 93827_270, Scaffold 68489_600, Scaffold 87490_633, Scaffold 87490_640, Scaffold 82042_3387, C35069468_1916, and Scaffold 93942_1089 were found to be associated with salt tolerance.

8.8 Genomics-Aided Breeding for Tolerance Traits

With the advent of new technologies, biological studies are touching new heights. Various genomics approaches like marker-based genotyping, genome resequencing, transcriptome, proteome, and methylome based studies have been applied extensively in many crops like rice, wheat, maize, etc. for deciphering multiple traits. The debatable issue of biosafety in use of transgenic crops for commercial cultivation suggest that molecular marker aided conventional methods of breeding may be the main short-term option for increasing productivity.

8.8.1 Structural and Functional Genomic Resources Developed

Whole genome sequencing: most legume species haven't been studied intensively in genome sequencing and breeding programs. Soybean was the primary leguminous crop to be sequenced (Schmutz et al. 2010). After this many other leguminous crops like pigeon pea (Varshney et al. 2012), chickpea (Varshney et al. 2013), common bean (Schmutz et al. 2014), mungbean (Kang et al. 2014), *Vigna angularis* (Kang et al. 2015), peanut (Bertioli et al. 2015) and cowpea (Lonardi et al. 2019) are sequenced. The genus *Vigna* includes legume crops like cowpea, mungbean, and azuki bean, also as >100 wild species. Varieties of the wild species are highly tolerant to severe environmental conditions including high-salinity, acid or alkaline soil; drought; flooding; and pests and diseases (Chankaew et al. 2014, Tomooka et al. 2014, and Yoshida et al. 2016). These features of the *Vigna* make it an honest target for the investigation of genetic diversity in adaptation to stressful environments; however, an absence of genomic information has hindered such research during this genus.

Once the entire genome sequence is accessible, it will produce a plethora of useful information like the number of genes present, repeat sequences and their position, copy number variation of a gene, etc. furthermore it will be used as a reference for genome and transcriptome assembly. As mentioned, *Vigna* species are still not much explored for genome sequencing and resequencing. Only a few species like *Vigna mungo, V. radiata, V. unguiculata, V. anguilaris, and V marina* are sequenced till date. *V. Marina* has the flexibility to tolerate salt stress, hence it's got a good potential to contribute salt tolerance genes in *Vigna* breeding but unfortunately, it is still underutilized in breeding programs. Its draft genome sequence is obtainable with 365.6 Mb size having 68,731 scaffolds. A complete of 35,448 SSRs and 50,670 genes were identified within the genome (Singh et al. 2019). Pootakham et al. (2020) reported the preliminary assembly of urdbean, contained 12,228 contigs which were further improved to 11 pseudomolecules covering 499 mb using advanced techniques. Comparative genomics analyses supported sequence information from single-copy orthologous genes revealed that urbean (*Vigna radiata*) diverged about 2.7 million

years ago. The reference urdbean genome revealed a lower proportion of repetitive elements within the urdbean genome, unlike other *Vigna* species.

Chloroplast genome sequencing; The cp genome of upper plants is a circular molecule of double-stranded DNA and is highly conserved in terms of its structure and its gene content with the dimensions starting from 72 to 217 kb containing ~130 genes, depending on the plant species (Sugiura 1995). A pair of huge inverted repeats (IRs) that are usually 10–28 kb long divides the genome into one large single-copy (LSC) region and one small single-copy (SSC) region. To date, only few complete legume chloroplast genomes are reported like *Cicer arietinum* (Jansen et al. 2008), *Trifolium subterraneum* (Cai et al. 2008), *Phaseolus vulgaris* (Guo et al. 2007a, b), *Lotus japonicus* (Kato et al. 2000), soya bean (Saski et al. 2005), bushand *Medicago truncatula.* Chloroplast genomes of Fabaceae family members are known to own undergone more rearrangements than other angiosperms. Among the *Vigna* species chloroplast genome of *V. mungo* is sequenced which reveals 108 unique genes and 19 of which are duplicated within the IR. Of these, 75 are predicted protein-coding genes, 4 ribosomal RNA genes, and 29 tRNA genes (Tangphatsornruang et al. 2010). Availability of complete chloroplast genome sequence could be highly useful in delineating the phylogenetic complexity among the legumes.

Some of the workers have attempted to develop functional genomic resources, Win et al. (2011) evaluated 12 *Vigna* genotypes for three different concentrations of salt (75, 150, and 225 mM) at the seedling stage for salinity-associated parameters and also performed sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Generally, plant height, leaf number, shoot and root length, chlorophyll content, shoot and root fresh weight, shoot and root dry weight, leaf area, and relative water content decreased with increasing salinity. In SDS PAGE they found some unique banding patterns at 45 kilodaltons (kDa). Tan et al. (2016) performed a comparative transcriptome profiling between tolerant and resistant lines of *Vigna unguiculata subsp. sesquipedalis* and the functional classification of the DEGs in two cultivars indicated that RNA, protein, signaling, stress, and hormone metabolism were five major groups. In the RNA group, analysis of TFs in the DREB subfamily revealed the existence of the ICE1-CBF3-COR cold-responsive cascade in asparagus bean, which is similar to Arabidopsis.

Butsayawarapat et al. (2019) performed the molecular characterization of mechanisms controlling waterlogging tolerance using two *zombi pea* [*Vigna vexillata* (L.) A. Rich] varieties with contrasting waterlogging tolerance and found that in contrast to the sensitive variety, Under waterlogging, the tolerant variety was able to grow, retain chlorophyll, form lateral roots, and produce aerenchyma in the hypocotyl and taproots. Further comparative transcriptome analysis of root tissues revealed that glycolysis and fermentative genes were strongly upregulated in the sensitive variety, but not in the tolerant one. The genes involved in auxin-regulated lateral root initiation and development, on the other hand, were only expressed in the tolerant type. Under waterlogging, the resistant variety's cell wall alteration, aquaporin, and peroxidase genes were all highly induced. Pan et al. (2019a, b) found 216 root-derived and 127 leaf-derived differentially expressed genes (DEGs) under salt stress between the two cultivars of *Vigna unguiculata* ssp. Sesquipedalis by using transcriptomic

approaches. Of these DEGs, thirteen were assigned to six transcription factors (TFs), including AP2/EREBP, CCHC (Zn), C2H2, WRKY, WD40-like, and LIM. GO analysis indicated four DEGs might take effects on the "oxidation–reduction", "transport" and "signal transduction" process.

Comparative transcriptome analysis was performed by Kumar et al. (2020a, b) between K-851 (drought tolerant) and PDM-139 (drought susceptible) lines of mungbean and concluded that the majority of DEGs were mapped to phytohormone signal transduction, carbon metabolism, sugar biosynthesis, and hormone signaling. They also concluded that ABA signaling, flavonoid biosynthesis, greater accumulation of osmolytes, etc. Helps, in developing a good root system to absorb more water in tolerant line. Similarly, Zhu et al. (2020) also conducted comparative transcriptome profiling of drought-tolerant and susceptible lines of Adzuki bean and concluded that all of the significant DEGs were involved in primary or second metabolism, plant hormone signal transduction, transcript or translation processes, the ubiquitin–proteasome system, transcription factor, transporters, and so on, which are also in congruence with previous studies (Zhang et al. 2020).

8.8.2 GWAS and Genomic Selection

To expand mungbean and urdbean cultivation into newer ecological niches, to meet ever-increasing demand requires breeding varieties tolerant to different abiotic stresses. It requires an understanding of genetic variation present in the germplasm. The gradual erosion of the genetic diversity of cultivated species will result in narrow genetic base of the crop. Hence evaluating the germplasm diversity and genetic basis of different traits should be of top priority. Molecular breeding approaches of using genetic markers for the selection of favourable genotypes will speed up breeding programs. The use of genome-wide association studies (GWAS) to discover the genetic variation in germplasm has proved to be an effective method. Genome-Wide Association Studies (GWAS) is a powerful method to associate agronomic traits to the genes that regulate them. GWAS studies help in assessing genetic diversity, population structure, Linkage disequilibrium (LD), and capability of using large and diverse germplasm panels to map/identify alleles associated with important agronomical traits using a high-throughput SNP genotyping platform.

DNA molecular markers help in mapping quantitative trait loci (QTL). QTL mapping necessitates the development of a segregating population resulting from the cross of two contrasting parents with respect to the traits of interest. If the target gene is contrasting, the QTL can be identified. As a result, the parental lines must be carefully selected, as the trait of interest must have a segregation pattern in the progeny. The number and type of DNA markers used in QTL mapping are determined by the number and type of markers used. The GWAS has several advantages over QTL mapping, including better resolution in locating QTLs controlling traits of interest and the ability to identify novel and superior alleles with greater precision.

It takes into account historical recombination. GWAS can detect certain polymorphisms in a gene that are responsible for the difference in a phenotypic trait between two individuals (Palaisa et al. 2003). GWAS has expanded, because of substantial advancements in DNA sequencing technologies, has helped the identification of a large number of molecular markers such as SNPs. In the mungbean, many workers have undertaken GWAS studies. Breria et al. (2019) identified loci associated with seed coat lustre (SCL) utilizing SNP markers. Their work resulted in identifying two loci in chromosome 5 significantly associated with SCL. Wu et al. (2020) worked on Genome-Wide SNP Identification and Association Mapping for Seed Mineral Concentration in Mung Bean. Noble et al. (2018), Characterized Linkage Disequilibrium and Population Structure in a Mungbean Diversity Panel which constitutes a valuable resource for genetic dissection of important agronomical traits to accelerate mungbean breeding. Sokolkova et al. (2020) investigated the genetic basis of variation in a number of important traits, using the mungbean mini-core series.

8.9 Recent Concepts and Strategies Developed

It has become an important tool in crop improvement programs and the advancement of CRISPR (Clustered regularly interspaced short palindromic repeats)/(cas9) CRISPR associated protein 9, has significantly speeded up crop crop breeding (Dita 2006; Muehlbauer et al. 2006). CRISPR/cas9 being used to obtain transgenic lines for abiotic stress tolerance in crops (Nadarajah and Kumar 2019). Number of microRNAs has been identified that are involved in a wide range of abiotic stress tolerance using CRISPR/cas9 (Qi et al. 2013; Yang et al. 2013; Bustamante et al. 2018).

8.10 Genetic Engineering for Tolerance Traits

Transgenic technology is specifically required when the desired gene is not present in the cross-compatible germplasm. However, the ongoing debate on biosafety and ethical issues involving the use of transgenic crops for commercial cultivation slowed down the efforts to develop improved cultivars through transgenic technology. Sainger et al. (2015) developed an efficient rapid and direct multiple shoot regeneration system amenable to Agrobacterium-mediated transformation from a primary leaf with the intact petiole of recalcitrant Urdbean (*Vigna mungo*). Similarly, Mekala et al. (2016) optimized the Agrobacterium-mediated genetic transformation of shoot tip explants in mungbean (*Vigna radiata* (L.) Wilczek). Chen et al. (2016) overexpressed *V. radiata VrDREB2A* into Arabidopsis and found that positive transgenic lines were tolerant to high salt concentration and drought stress indicating that this gene has a good potential for developing abiotic stress-tolerant mung bean and related plants. Mishra et al. (2014) conducted an experiment to overexpress a mungbean vacuolar Na^+/H^+ antiporter gene (*VrNHX1*) ectopically and found that it leads to

increased salinity stress tolerance in transgenic *Vigna unguiculata* L. Walp. They found that under salt stress conditions, T_2 transgenic 35S:VrNHX1 cowpea lines exhibited higher tolerance to 200 mM NaCl treatment than wild-type. Furthermore, T_2transgenic 35S:VrNHX1 lines accumulated more [Na^+] in roots and maintained a higher K^+/Na^+ ratio in aerial sections under salt stress than wild type. Transformation of codA gene, for an osmoprotectant, glycine betain biosynthesis in mungbean conferred resistance against abiotic factors like salinity and drought (Baloda et al. 2017). Bhomkar et al. (2008) identified that overexpression of the *Glyoxylase I* gene using using a novel *Cestrum Yellow Leaf Curling Virus* (*CmYLCV*) promoter was directly correlated with their ability to withstand salt stress. Recent researchs on urdbean shown that altered overexpression of *ALDRVX4* gene confers multiple abiotic stress tolerance (Singh et al. 2016a, b). The *ALDRVX4* gene belongs to aldo–keto reductase superfamily, which performs reactive carbonyl detoxification and plays an important role in osmoprotection.

Surekha et al. (2014) found that expression of the mutagenized *Vigna aconitifolia P5CSF129A* gene in transgenic pigeon pea enhances proline accumulation and salt tolerance, hence may serve as a potential for developing salinity tolerance in *Vigna* species. Sahoo et al. (2016) reported overexpression of an Arabidopsis *NHX1* (*AtNHX1*) in transgenic mungbean plants conferred enhanced salt tolerance. In their experiments, T_2 transgenic lines under salt stress accumulated higher K^+/Na^+ in the aerial parts and higher [Na^+] in roots than wild type plants (WT). Moreover, the T_2 transgenic lines showed under NaCl treatment reduced membrane lipid peroxidation and HO_2 and O_2 accumulation, higher levels of antioxidant enzyme activity, and increased accumulation of proline and ascorbate than WT.

Rout et al. (2020) found that overexpression of *ICE1* gene in transgenic mungbean plants resulted in cold-tolerance at the seedling stage when compared to non-transformed plants. Cold stress signaling activated with the CCAATT motif binding factor (CBF) which in turn induce the activity several cold stress genes. Transcription factors, Inducer of cbf Expression 1 (*ICE1*), present in upstream of signaling pathway regulate *cbf* genes during cold stress conditions (Chinnusamy et al. 2003). During cold stress, transcription factors present in cell recognizes the CBF promoters and induces CBF expression (Gilmour et al. 1998). Over expression of cold responsive transcription factor, ICE1 in mungbean plants conferring in cold-tolerance at the seedling stage had significantly increased germination and root and shoot growth at 10–14 °C.

Transgenic studies in mungbean and Urdbean, (Table 8.15) are very limited in number legumes since they are highly recalcitrant and genotype specific requirements in vitro conditions. The regeneration potential remained very low. While, very few good results were reported by using cotyledonary node and shoot tip as an explant. Till date very few achievements obtained in successful recovery of transgenics in mungbean following genetic transformation protocols. Candidate genes explored for imparting drought tolerance in *Vigna* species has been mentioned in Table 8.16.

Table 8.15 Comprehensive list of transgenes encoding functional proteins or stress-adaptive compounds for direct protection from salinity stress tolerance

Gene transferred	Product	Source organism	Target plant	Transgene effect	Reference(s)
Glycine betaine synthesis					
codA	choline oxidase	*Agrobacterium tumefaciens*	*Vigna radiata*	Shoot regeneration from cotyledonary node (without cotyledons) under stress condition	Baloda et al. (2017)
Glyoxalase pathway					
GLYI	Glyoxalase-I	*Brassica juncea*	*Vigna mungo*	Enhanced salt tolerance	Bhomkar et al. (2008)
Other stress-responsive genes					
AtNHX1	Na^+/H^+ antiporter	*Arabidopsis thaliana*	*Vigna radiata*	Improved tolerance under salt tress to ionic, osmotic, and oxidative stresses	Kumar et al. (2017)
VrNHX1	Na^+/H^+ antiporter	Mungbean	*Arabidopsis thaliana*	Enhanced tolerance to salinity	Mishra et al. (2014)
ALDRXV4	Reduction of carbonyl metabolites	*Agrobacterium tumefaciens*	*Vigna mungo*	Osmoprotection and detoxification of the reactive carbonyl species under salt tress	Singh et al. (2016)

Table 8.16 Candidate genes explored for imparting drought tolerance in *Vigna* species

Crop	Gene transferred	Function	References
Mungbean	*VrbZIP*	Drought-responsive gene	Wang et al. (2018)
	codA	Improve abiotic stress tolerance	Baloda et al. (2017)
	VrWRKY	Enhance abiotic stress tolerance	Srivastava et al. (2018)
Cowpea	*VuPLD1, VuNCED1, CPRD8, CPRD12, CPRD14, CPRD22*	ABA-biosynthesis	Muchero et al. (2010)

8.11 Role of Bioinformatics as a Tool

Attempts have also been made to develop a few webserver/repositories (Table 8.17) containing the genomics and related information of selected *Vigna* species, like *Vigna* Genome Server (Vig GS) which incorporates annotated exon-intron structures, along with evidence for transcripts and proteins, visualized in GBrowse (Sakai et al. 2016).

Cowpea Genespace/Genomics Knowledge Base (CGKB), developed by Chen et al. (2007), is an annotation knowledge base developed under the Cowpea Genome Initiative which is based on information derived from 298,848 cowpea gene space sequences (GSS) isolated by methylation filtering of genomic DNA. The CGKB consists of three knowledge bases: GSS annotation and comparative genomics knowledge base, GSS enzyme and metabolic pathway knowledge base, and GSS simple sequence repeats (SSRs) knowledge base for molecular marker discovery.

Table 8.17 List of genomics data base

S. No.	Name of data base	Source link/URL
1	Next Gen Seek	http://nextgenseek.com
2	Kevin's GATTACA World	http://kevin-gattaca.blogspot.com
3	In Between Lines of Code	http://flxlexblog.wordpress.com
4	Next-Gen Sequencing	http://nextgenseq.blogspot.com
5	Core Genomics	http://core-genomics.blogspot.com
6	RNA-Seq Blog	http://www.rna-seqblog.com
7	Next Generation Technologist	http://www.yuzuki.org
8	Blog @ Illumina	http://blog.illumina.com
9	Bits of DNA	http://liorpachter.wordpress.com/seq
10	Journal of Next Generation Sequencing & Applications	http://www.omicsonline.org/nextgenerationsequencing-applications.php
11	Omics! Omics!	http://omicsomics.blogspot.com
12	PlantGDB	www.plantgdb.org/MtGDB/
13	LIS—Legume Information System	http://legumeinfo.org/gbrowsecajca1.0
14	Phytozome 10.2	http://phytozome.jgi.doe.gov/commonbean
15	Legume Information system	http://cicar.comparative-legumes.org/
16	Mungbean Genome Jbrowse	http://plantgenomics.snu.ac.kr/
17	Adzuki bean Genome Jbrowse	http://plantgenomics.snu.ac.kr/
18	PlantGDB	
19	Lotus japonicus genome assembly build 2.5	http://www.kazusa.or.jp/lotus/
20	PlantGDB	www.plantgdb.org/MtGDB/
21	Phytozome 10.2	http://phytozome.jgi.doe.gov/soybean

8.12 Conclusions and Future Prospects

Mungbean and urdbean are short-duration crops that are warm temperate or tropical in nature. They can be grown across different seasons, locations, and soil types; therefore they are also exposed to a variety of biotic and abiotic challenges. These crops have a distinctive position in cropping systems because of diversified uses and are of great significance in sustaining productivity in cereal-based agriculture by fixing atmospheric nitrogen through symbiotic association with Rhizobium bacteria. Breeding for photo- and thermo-period insensitivity, tolerances to moisture and temperature extremities, and tolerance to salinity are some of the major abiotic stresses affecting their versatility and climate resilience. Most of the breeding efforts to date concentrated more on biotic stresses and yield; hence research should be more prioritized towards breeding for abiotic stress tolerance. More efforts should be made on pre-breeding activities, trait discovery, and new plant types for different abiotic stresses. There is also a need to understand the genetic, physiological, and biochemical basis of abiotic stress tolerance and their interactions. Low heritability of traits conferring abiotic stress tolerance, due to involvement of polygenes, and their complex interactions requires molecular interventions. Recent advancements in gene manipulation techniques, transgenic, comparative, and functional genomics tools must be deployed to improve the crop productivity.

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Chapter 9
Genomic Designing Towards Development of Abiotic Stress Tolerant Grass Pea for Food and Nutritional Security

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Abstract Grass pea (*Lathyrus sativus* L.) is a climate-resistant underused cool season legume crop grown in marginal rainy regions of sub-Saharan Africa, South Asia and the Mediterranean region, where it is grown for food as well as forage. In the face of climate change and global warming, grass pea is gaining popularity as a more water and input efficient crop with the unique ability to improve soil fertility while emitting little carbon. With deep penetrating root system, grass pea possesses enormous genetic potential for drought, salt and flood tolerance as well as for surviving under waterlogged condition of rice fallow niches. Despite of its immense potential grass pes is still orphan in terms of genetic and genomic resource. Because of the stigma associated with the presence of β-ODAP responsible for crippling disease, grass pea cultivation has declined in the recent decade. New research has revealed grass pea's potential as a functional food with presence of health-promoting nutraceutical like homoarginine. Concentrated breeding efforts in grass pea have developed

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30 improved varieties with low ODAP content for their cultivation in diverse agro-ecologies. Molecular research is still lagging behind and renewed research is needed for strengthening molecular breeding program in grass pea. Availability of draft genome sequencing is a significant step forward towards the application of genomic-assisted breeding in this crop. Grass pea is the golden pulse of future for diversification of more energy and input intensive cereal-based cropping systems which are not long term viable. This chapter focuses on the redesigning of abiotic stress tolerant grass pea and highlights the research gaps that need to be contemplated to make it as a "wonder crop" of future.

Keywords Grass pea · ODAP · Climate resilience · Abiotic stress · Genetic improvement · Genomic resources

9.1 Introduction

Grass pea (*Lathyrus sativus L.*) (diploid, $2n = 14$) belongs to the tribe Vicieae and family Fabaceae, acknowledged as one of the ancient crops, which has been domesticated long ago in in the Balkan peninsular region (Campbell 1997). This potential legume is a geographically versatile crop sharing useful genes for important physiological processes as well as for defence mechanisms. The name "Lathyrus" comes from the Greek word "lathyros", which shows something exciting in relation to the aphrodisiac properties attributed to grass peas (Loudon et al. 1855). It is well-known as khesari or teora in India and Bangladesh, Matri or Mattra in Pakistan, Almorta in Spain, chickling vetch or Indian vetch in United Kingdom and United States of America, Guaya in Ethiopia, cicerchia in Italy, and Gilban in Sudan. Grass pea is an underutilized, neglected pulse mainly grown as both food and feed in the marginal land by resource poor farmers of South East Asia including India, Nepal, Bangladesh, Afghanistan, Pakistan, China, in Mediterranean region, Australia, as well as African countries like Sudan and Ethiopia with minimum inputs and acts as the ultimate source of energy during climatic vagaries like drought, famine etc. (Campbell 1997; Vaz Patto et al. 2006; Mahapatra et al. 2020). As per the recent report the total area under grass pea cultivation is roughly 0.70 million ha and the production is 0.79 million tons (Kumar et al. 2020) which was previously 1.50 million ha with an annual yield of 1.20 million tons (Kumar et al. 2013; Sammour 2014). The grass pea growing area is mainly concentrated in South Asia and Sub-Saharan Africa. Interestingly, productivity has been raised to 1130 kg/ha from the earlier productivity of 750 kg/ha (Kumar et al. 2020) due to popularization and cultivation of high yielding improved varieties with good agronomic practices. India is the world's top producer and consumer of grass peas. In Indian subcontinent this food legume mainly finds its place in marginal area and it is a good candidate for rice fallow ecosystem as a 'utera' crop having further possibility of increment of area and productivity (Maji et al. 2019).

Grass pea is a crop of future and insulated against almost all abiotic and biotic stresses. In drought as well as in flood condition, grass pea is the potent candidate with stable performance among the food legumes (Zhelyazkova et al. 2016). With deep penetrating root system, grass pea is also capable of withstanding salinity, nutrient imbalance of the poor soil and heavy metal toxicity. In India and Bangladesh under common 'Utera' cultivation the grass pea seeds are generally broadcasted in between the straws of the standing rice crops before its harvesting to thrive in the clayey soil with excessive moisture. Being a leguminous crop, grass pea is capable of fixing atmospheric nitrogen and the nitrogen fixation rate registered is 124 kg/ha/year (Schulz et al. 1999). This food legume is also having better resistance against most of the fungal and bacterial diseases as well as pest infestation as compared to other pulse legumes (Vaz Patto et al. 2006).

9.1.1 Nutrients Composition

In mankind history grass pea is considered as the life saviour food despite of the stigma of 'neurolathyrism' or presence of neurotoxin associated with it as because when natural disasters wiped out all other foodstuffs, grass pea was the lifeline of million people in times of food shortage (Girma and Korbu 2012; Lambein et al. 2019; Sarkar et al. 2019). Grass pea seeds can be used as a food in various forms ranging from split grain as dhal or roasted whole seeds, boiled whole seeds, flour and also for the preparation of sauce and local drinks (Lambein et al. 2019). In India and Bangladesh, the young shoots, seeds as well as green pods are also consumed as fresh vegetables. Grass pea is the cheapest source of protein of vegetarian and vegan diet who cannot afford animal protein with good amino acid balance and having very low fat and starch in its grain (Hillocks and Maruthi 2012). In grass pea, enough variability is observed regarding its protein content ranging from 17.7 to 49.0% which is greater than most of the food legumes except soybean (Sammour et al. 2007; Rizvi et al. 2016; Barpete et al. 2021). The protein of grass pea is highly balanced and contains almost all 17 essential amino acids in which the most prevalent one is globulin (60% of the total proteins) followed by albumins accounting 30% of the total protein. Previous study reported the total amino acid content in grass pea is about 19.69–23.48 g/100 g seeds (Arslan 2017). The lysine content of grass pea is higher than the cereal but like other food legumes it is also deficient in Sulphur containing amino acids i.e. cysteine and methionine (Yan et al. 2006; Pastor-Cavada et al. 2011). Interestingly, a grass pea mutant has been detected having 63% more methionine than its parent (Asnake 2012). Grass pea has a very good nutrient profile including macro- and micronutrients. Grass pea germplasms have phosphorus in the range of 380.4–511.6 mg/100 g and calcium in the range of 131.6–200.1 mg/100 g. Grass pea seeds contain substantial amount of potassium (8.3–10.8 g/kg). Beside proteins and major nutrients grass pea seeds contain approximately 41% carbohydrate, 2.7% fat, 17% total dietary fiber with an energy value of 362.3 kcal/kg energy (Aletor et al. 1994). The fatty acid content of grass pea seed is beneficial and healthy for human diet as 58% of the fatty

acids in grass pea are polyunsaturated with good proportion of linoleic acids and meager amount of oleic, linolenic acids which ultimately increase the shelf-life of the foods prepared from grass pea seeds (Grela et al. 2010). The number of various unsaturated fatty acids in total lipids is 1.670.18 g/100 g, with the essential PUFA like linoleic, linolenic and linolenic acids being abundant. This food legume also contains substantial amount of vitamin C (13.50 ± 0.30 mg/100 g), glutathione (15.90 ± 0.10 mg/100 g) which is basically composed of three amino acids, vitamins from A family like vitamin A_1 or retinol (34.9 μg/kg), β-carotene (323.3 μg/kg), different members of water soluble vitamin B like thiamine or vitamin B_1 (4.60 mg/kg), riboflavin or vitamin B_2 (2.30 mg/kg), nicotinic acid or vitamin B_3 (16.40 mg/kg), pantothenic acid or vitamin B_5 (18.40 mg/kg), pyridoxine or vitamin B_6 (5.80 mg/kg), and folic acid or vitamin B_9 (5.40 mg/kg) (Tamburino et al. 2012). Additionally, it had the highest flavonoid concentration and antioxidant activity (Sarmento et al. 2005). L-homoarginine is one of the first odd non-protein amino acids discovered only in grass pea to be employed as a substrate for the regulated production of nitric oxide and to play an important role in the treatment of cardiovascular illness as well as suppression of cancer tumour growth. (Rao et al. 1963; Lambein 2000; Jammulamadaka et al. 2011; Van Wyk et al. 2016). As a result, grass pea is considered as a good example of a potential "functional food" (Singh and Rao 2013). Beside nutritional factor, grass pea contains some antinutritional components like phytate which lowers the mineral bioavailability, notably the absorption of Fe and Zn (Sandberg 2002). In grass pea, condensed tannins ranged from 0.89 to 5.18 g/kg dry matter. Grass pea exhibits a high amount of trypsin inhibitor, with trypsin inhibitor activity values ranging from 15.53 to 18.99 TIU/mg.

9.1.2 ODAP Content a Major Bottleneck Towards Grass Pea Promotion

The major constraint towards popularization of grass pea is due to the presence of β-ODAP or b-*N*-oxalyl-amino-L-alanine (BOAA) which causes neurolathyrism, a non-reversible neurological disorder in humans and animals caused due to regular consumption of grass pea (Lambein and Kuo 2009; Vaz Patto and Rubiales 2014). β-ODAP along with L-homoarginine are categorized as the major fraction of the free non-protein amino acids in grass pea seeds (Zhao et al. 1999). Due to the stigma associated with β-ODAP, the grass pea cultivation has been banned for a long time. The grass pea toxin ODAP generally presents in two isomeric forms (α and β) where β forms are most abundant accounting 95% of the total ODAP (Bell and O'Donovan 1966; De Bruyn et al. 1994). Despite of bad reputation of β-ODAP recently a patent has been granted for using β-ODAP as a hemostatic agent during surgery to a Chinese group (Lan et al. 2016).

Meager research is carried out to detect the biosynthetic pathway of ODAP and identify the putative genes associated with ODAP biosynthesis. It was observed

that ODAP is unanimously present in all plant parts but the concentrations in leaf and embryo are high during vegetative and reproductive stage respectively (Barpete 2015). It was hypothesized that ODAP content in grass pea is linked to total free nitrogenous chemicals, and that nitrogen and phosphate are the most important nutritional variables regulating neurotoxic concentration under field conditions. Previous studies have shown that nutritional deficiencies of cysteine and methionine may worsen the neurotoxicity of ODAP which suggested that ODAP biosynthesis is somehow related with nitrogen and sulfur metabolism (Xu et al. 2017).

Grass pea genotypes exhibited wide range of variations regarding ODAP content ranging from 0.02 to 2.59% depending on genotype and environmental interactions and genetic structure (Ramanujam et al. 1980; Fikre et al. 2011; Kumar et al. 2011, 2013). Earlier reports suggested that various abiotic stresses like drought and heat, nutrient imbalance specially zinc depletion in the soil trigger ODAP biosynthesis through influencing plant's osmotic potential (Piwowarczyk et al. 2014; Liu et al. 2017). However, the exact physiological and molecular mechanisms underlying ODAP content in grass pea remain unexplored till date which seeks urgency to unveil the biochemical pathway of ODAP biosynthesis in grass pea.

9.1.3 Limitations of Traditional Breeding Efforts

Grass pea is considered as orphan crop in terms of cultivation as well as research point of view as this is the crop of interest for the poor farmers and mainly cultivated in marginal land with minimum inputs. As compared to cereals and other cash crops, grass pea is not the subject of interest of the international scientific community and there is paucity of scientific documentation as well as research funding towards converting this food legume from orphan to mainstream crop. The major bottlenecks towards grass pea improvement are due to the ban imposed in several countries across the globe for the presence of phytotoxin ODAP, the pollination nature of this crop, large genome size, restrained economic importance in the global market and dearth of formal seed supply chains to provide good quality seeds to farming community (Cullis and Kunert 2017). In grass pea, the outcrossing percentage is up to 30% considering the flower structure and color, entomophily and growing condition so it is a tedious job to maintain the purity of the cultivars which are mainly pure line varieties (Chowdhury and Slinkard 1999). The outcrossing percentage is less in white flowered cultivars in comparison to blue, pink and crimson color and in large sized flower the outcrossing percentage is more (Kiyoshi et al. 1985; Rahman et al. 1995). Another major issue of limited genomic resources available in grass pea breeding program is because of its large genome size. The draft genome sequence of 'LS007', a European grass pea accession, was recently published, with an estimated genome size of 6.3 Gb which is over ten times as much as chickpeas and one-and-a-half times as much as lentils and peas (Emmrich et al. 2020). In grass pea the major research was concentrated on either chromosomal biology or about the plant toxin ß ODAP and its effect on vertebrates as food or fodder with limited focus on developing genetic and genomic

resources. With systematic breeding efforts rendered by several national and international institutes, a large number of improved cultivars with low ß ODAP content (0.1%) have been developed (Abd El Moneim et al. 2001; Lambein et al. 2019). But development of new marker systems, initiation of genome-wide association studies (GWAS) and marker assisted selection (MAS) based improvement program, deployment of next generation sequencing (NGS) based genomic resources and reverse genetic approaches like targeting induced local lesions in genomes (TILLING) and Eco-TILLING need to be strengthened in grass pea breeding program for tapping genomic resources in this underutilized food legume.

9.2 Grass Pea with Unique Traits to Counteract Abiotic Stresses

The grass pea is a Neolithic legume that has been cultivated for millennia and has spread across three continents with unique feature to defeat climatic aberrations (Yang and Zhang 2005; Lambein et al. 2019). More than 100 million resource poor people from drought-prone areas of South Asia, Middle East and East Africa depend on this food legume for their energy source as well as for fodder purpose since it is a low input crop and relatively resistant to all kind of biotic and abiotic stresses (Abd El Moneim et al. 2000). Many of these areas experience extreme weather and traditionally cultivate pulse crops in rainfed environment where farmers are resource poor, ignorant of improved agricultural practices, and with lack of irrigation facilities, rendering pulse crops unproductive and highly vulnerable to abiotic stresses that can result in yield losses of up to 50% (Vez Patto et al. 2006; Maji et al. 2019). Grass pea exhibits manifold tolerance to climatic vagaries like temperature extremities, drought, salinity, submergence, mineral deficiency and act as a potential genetic resource (Ali et al. 2000; Siddique et al. 2001; Ahmed et al. 2014; Lambein et al. 2019). Grass pea has unique rhizofiltration capacity to exclude out heavy metal toxicity (Brunet et al. 2008; Marzban et al. 2017). In the following section the genetic potential of this crop as a "climate resilient" will be highlighted towards boosting up the economy of marginal land resources.

9.2.1 Root Characters of Grass Pea

Grass pea having incredible capability to withstand strident environmental conditions and acknowledged as unique source of genes of interest. In most of the grass pea growing belts this crop is being cultivated for reclaiming marginal land with an alternative of cereal and also to meet the protein and calorie demand of the society. Grass pea is known to have a very hardy and deep penetrating root system thus enables to counteract salinity, flood and drought stress (Campbell 1997). Moreover,

deep penetrating root system also facilitate better nutrient and water mobilization in limiting condition.

9.2.2 Grass Pea Defying Temperature Extremities

Grass pea is one of the unique pulses that can be cultivated in both high altitude of India, Ethiopia as well as in plains of Gangetic Alluvial zones of India and Bangladesh (Girma and Korbu 2012). This flexibility explains grass pea's capacity to survive climate extremites such as heat and cold. Although, grass pea is a cool season food legume therefore, temperature elevation more than 30 °C extremely affects reproductive phenology. During heat stress, the vegetative phase diminishes which triggers early occurrence of reproductive phase. Pollen viability and stigma receptivity drastically reduces which ultimately retards quality and production. Earlier report stated that, when grass peas were exposed to 55 °C for 48 h, they developed alterations in flower color as well as meiotic anomalies that resulted in pollen sterility (Kumar and Tripathi 2009). In comparison to other food legumes grass pea is almost hardy against all sorts of environmental perturbations through inducing protein thermal stability as well as antioxidative enzyme activity, overexpressing genes related with antioxidative enzymes. During selection of heat tolerant genotypes, seed weight, pods number, yield as well as 50% flowering are the selection indices that should be considered. Grass pea exhibits wide variation in terms of heat tolerance. Red pea (*L. cicera*) which is the wild progenitor of grass pea can withstand heat stress of upto 58 °C during reproductive phase (Robertson and Abd El-Moneim 1995; Icoz et al. 2014). Strikingly, one Portuguese accession of *L. ochrus* was identified to withstand cold stress through escape mechanism (Abd El-Moneim and Cocks 1993). Further intensive efforts are needed to unveil the mechanism behind overcoming heat stress as well as identifying resistant sources followed by tapping of the genes of interest towards developing heat and cold tolerant cultivars in this food legume.

9.2.3 Grass Pea with Unique Traits and Trails of Drought Tolerance

Among various abiotic stresses, drought is considered as one of the most detrimental stresses to plant growth and development. Grass pea is mainly cultivated in arid and semiarid zones where drought is a frequent phenomenon which negatively influence soil water potential resulting poor seed germination, reproductive growth, yield and harvest index (Polignano et al. 2009; Palta et al. 2012; Bhat et al. 2020). There is paucity of information on the genotypic variability of grass pea species under moisture stress. The *L. cicera* was detected as stress resistant, whereas the *L. aphaca* was the susceptible one. Grass pea has acquired various morphological drought resistance

qualities, such as thin leaves, winged borders on stems for reduction of transpirational losses and maintaining a higher water level for photosynthesis during moisture deficit (Jiang et al. 2013; Lambein et al. 2019). Shoot and root architecture, as well as distribution pattern and ecophysiological measures like water extraction, are the most important features that indicate drought resistance in grass pea (Siddique et al. 2001). The root architecture of grass pea is well developed, robust, and penetrating, with increased root depth and biomass that will facilitate efficient moisture uptake (Bultynck et al. 2004; Blum 2011; Franks 2013; Fenta et al. 2014; Koevoets et al. 2016). Earlier reports pointed out some adaptive mechanisms in grass pea to mitigate drought stress through modifying opening and closing of stomata, over accumulations of various osmoprotectants like proline and soluble sugars for improving water use efficiency coupled with upregulation of the genes related with various antioxidative enzymes like catalase (CAT), peroxidase, superoxide dismutase (SOD), glutathione peroxidase (GPX) for scavenging reactive oxygen species (Jiang et al. 2013). An interesting study conducted on grass pea using 20% polyethylene glycol 6000 (PEG) as drought stress revealed that hydrogen peroxide (H_2O_2) content was almost 1.5 folds increased upon polyethylele glycol (PEG) treatment while malondialdehyde (MDA) content was uplifted by 1.3 folds (Jiang et al. 2013). Additionally, the same study depicted that proline and soluble sugar contents increased by six folds and two folds, respectively in the PEG treated grass pea leaves. Among the enzymatic antioxidants, the SOD and CAT activities upregulated 2.3 and 1.5 folds, respectively while ascorbate peroxidase (APX) increased 2.2 folds and GPX increased about 1.5 folds in the PEG 6000 treated grass pea compared to the untreated one (Jiang et al. 2013). Further study highlighted increased MDA content in grass pea shoots treated with 17.5 mM PEG compared to the untreated one (Tokarz et al. 2021). Moreover, compared to the control plants the soluble sugar content increased in shoot tissues at 17.5 as well as 22 mM PEG treatment and only in the root tissues at higher PEG treatments while the accumulation of insoluble sugar was uplifted in the shoot tissues only at both the tested PEG concentrations (Tokarz et al. 2021). Proline content depicted higher accumulation under both the PEG treatments in root as well as shoot tissue while CAT activity was elevated only at 22 mM PEG treatment compared to the untreated plants. Strikingly some reports stated that under drought and heat stress the accumulation of β-ODAP content is increased which might be related with protecting glycolate oxidase activity from reactive hydroxyl radicals (Xing et al. 2001; Yang and Zhang 2005; Kumar et al. 2011). Although the effect of β-ODAP accumulation in abiotic stress tolerance in grass pea is yet obscure, this component of the research requires additional investigation.

An interesting study unraveled that several microRNA (miRNA) from grass pea are involved in drought stress signaling (Bhat et al. 2020). Gene expression in plants as well as in other eukaryotes are controlled by several factors and miRNA is one of them which is originated from an endogenous gene and regulate gene expression either through mRNA cleavage of target genes or through translational regulation (Akdogan et al. 2016). Limited study on drought tolerance mechanisms in grass pea limits inferences about physiological and molecular insights into drought tolerance (Jiang et al. 2013).

9.2.4 Unique Adaptation of Grass Pea in Flood Situation

Grass pea is a potential candidate crop in rice fallow niche which can prove the adaptation of this crop to excessive moisture (Abd El Moneim et al. 2001; Maji et al. 2019). Grass pea has evolved several adaptive mechanisms to withstand the poor oxygen availability during flooding and submergence. Among the various food legumes, grass pea is considered as tolerant to moderately susceptible against flood stress (Solaiman et al. 2007; Malik et al. 2015). It was observed that grass pea with large seed size has better germination capacity under submerged condition in comparison to other legumes. After germination, the root porosity is generally increased under excessive moisture which ultimately maintain the leaf chlorophyll and shoot nitrogen content (Malik et al. 2001). In South East Asia around 4 million ha land, grass pea is generally broadcasted into a standing rice crop as a "relay" or "paira" crop before the harvesting of paddy in excessive moisture condition to avoid tillage operation and to ensure germination using the residual moisture (Das 2000). Grass pea with a deep penetrating root system can withstand excessive water in rice fallow low-lying locations as well as severe drought. Earlier study confirmed that, under flood situation the cell death is restricted only in the root tip zone followed by accumulation of antioxidative enzymes to facilitate scavenging of reactive oxygen species (ROS) and protection of other root cells from oxidative damage (Zhou et al. 2016). Furthermore, grass pea develops lateral roots and aerenchyma, which minimizes oxygen use, aid oxygen transfer from shoot to root, and remove harmful by-products. (Zhou et al. 2016). Improved grass pea varieties with good biomass, as well as agronomic modification, are needed to boost the output and productivity of this crop as a food and feed under submerged condition of rice fallow niche towards paving the path for long-term sustainability and sustenance (Das et al. 2021).

9.2.5 Grass Pea Counteracting Salinity

Salt stress is another important abiotic stress causing biochemical and physiological changes in plants and ultimately hampers the growth and development of plant. According to the FAO/UNESCO World Soil Map, salty soils cover 397 million hectares, accounting for roughly 20% of world land and half of irrigated land (Silva and Geros 2009; Hussain et al. 2010). Grass pea is basically cultivated in arid and semi-arid zones of the World where the substantial area is affected with salt stress. Beside ion toxicity salinity also aggravate drought stress and induce generation of ROS within plant cell (Talukdar 2013; Gheidary et al. 2017). Grass pea germination and early seedling growth is not affected too much by mild salt stress (up to 100 mM NaCl stress) while further stress cause detrimental effects on plant growth (Piwowarczyk et al. 2016) as salt rises, the young seedling's ability to absorb water decreases, resulting in reduced cell division and seedling growth (Tsegay and Andargie 2018). Salinity has a negative impact on the reproductive stage and causes problems with

metabolite partitioning, resulting in a lower seed output (Rana et al. 2016). Earlier reports indicated that seed priming with gibberellic acid (GA_3) can improve germination and early seedling growth of grass pea in saline condition. Comparison of two salt treatments (50 and 100 mM NaCl) along with the untreated condition depicted that at 100 mM NaCl stress, proline and total phenol contents were increased only in the root tissues compared to other treatments. Similarly, the activities of enzymatic antioxidants like CAT and POD were mostly upregulated in root tissues among the tested genotypes at 100 mM NaCl stress (Piwowarczyk et al. 2016). Another study also depicted that the antioxidant activity upon exposure to salt stress were mostly enhanced in the root tissues compared to the shoot part (Tokarz et al. 2021). An interesting study revealed the mechanisms of toxic Na^+ ion distribution in grass pea plants upon salt stress exposure. Stems translocate Na^+ to the leaves under salt stress and carboxylation is reduced in leaves but increased in stem causing stem as the main part of assimilation during salt stress in grass pea (Tokarz et al. 2021). Root is the first zone that can sense salinity. Thus, mechanisms ensuring ion inclusion or compartmentation as well as overexpression of antioxidant enzymes activity in the root cells would be helpful for alleviating salt stress in grass pea (Hura et al. 2009; Chattopadhyay et al. 2011; Talukdar 2013; Piwowarczyk et al. 2016). Grass pea exhibited variation regarding sensitivity to salt stress. Germplasm from Mediterranean and Ethiopian region exhibit better adaptability under salt stress (Vaz Patto et al. 2006; Haileselasie 2012).

9.3 Genetic Resources of Resistance/Tolerance Genes Against Abiotic Stresses

9.3.1 Available Germplasms

In plant breeding programs, the response to selection is completely hinged on genetic diversity. The genus *Lathyrus* is diverse and bestowed with ~160 other species (Asmussen and Liston 1998; Vez Patto and Rubiales 2014). Grass pea genetic resources are being conserved globally by several international as well as national organizations which are available online at global portals—Genesys (https://www.genesys-pgr.org/) where information about 6,556 accessions have been displayed (Table 9.1). Around 26,066 accessions are included in the grass pea database created from the worldwide repository including duplication of some accessions. Among the various gene banks of different organizations, ICARDA holds the major accessions (4,457) at its breeding head quarter Beirut, Lebanon. Besides, the University of Pau in France (4,000), National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India (2,600), as well as the Plant Genetic Resources Centre in Bangladesh (1,116) also maintain a large diverse collection of *Lathyrus* germplasm. This collection consists of around 2,143 wild as well as accessions of crop wild relatives (CWR), with major share from *L. aphaca* (575) followed by *L. cicera* (337) which are the

Table 9.1 Grass pea Genetic Resources conserved Worldwide in the Genesys portal (data accessed through GENESYS Global Portal on Plant Genetic Resources, https://www.genesys-pgr.org, 2021–06-12)

S. no	No. of germplasm	Country	Institute/organization
1	4184	Syria/Morocco	International Center for Agricultural Research in Dry Areas (ICARDA)
2	2619	India	National Bureau of Plant Genetic Resources (NBPGR)
3	1841	Bangladesh	Plant Genetic Resource Centre (PGRC), Bangladesh Agricultural Research Institute (BARI)
4	1424	Chile	Instituto Nacional de Investigación Agraria (INIA)
5	1215	Ukraine	Ustymivka Experimental Station of Plant Production
6	1207	Russian Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry
7	1020	Australia	Australian Grains Genebank
8	949	USA	ARS-GRIN Pullman, ARS Ft Collins, Boyce Thompson Arboretum, Arizona, ARS National Arboretum, Washington D.C
9	840	Canada	Plant Gene Resources of Canada (PGRC)
10	515	Germany	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
11	429	Spain	Centro de Recursos Fitogenéticos (CRF) Instituto nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
12	1115	UK	Royal Botanic Gardens, Kew
13	949	USA	ARS-GRIN Pullman, ARS Ft Collins, Boyce Thompson Arboretum, Arizona, ARS National Arboretum, Washington D.C
14	704	China	National Gene bank, Beijing
15	155	Ethiopia	International Livestock Research Institute
16	47	Greece	Agricultural Research Center of Macedonia & Thrace
17	42	Italy	Universitá degli studi Perugia, CRA-Centro di Ricerca per
18	22	Portugal	Universidade da Madeira
19	14	Azerbaijan	Genetic Resources Institute

valuable sources of novel alleles for developing climate resilient varieties in grass pea for their adaptation in varied niches. In comparison to other food legumes, the breeding program in grass pea is limited due to narrow range of genetic variation, self-pollination and incompatibility with wild relative (Kumar et al. 2013).

9.3.2 Grass Pea Gene Pools

The genus *Lathyrus* with about 160 species and 45 subspecies (Allkin et al. 1986) is taxonomically classified in to 13 sections (Kupicha 1981). The genus *Lathyrus* included both annual and perennial species. Annual species include *Aphaca*, *Nissolia*, *Clymenum*, *Cicerula* and *Lathyrus* is mainly the perennial one (Asmussen and Liston 1998). The species are all found in the Old World, primarily in Europe, Near East and the North Africa, but even as far as India, Bangladesh and other South Asian countries. Harlan and De Wet (1971) established the gene pool idea to enable a better cataloging of crop plants and their CWRs. On the basis of crossability, the genus *Lathyrus* has been classified into primary, secondary and tertiary gene pools (Jackson and Yunus 1984; Yunus and Jackson 1991; Kumar et al. 2013). Besides, the gene pool described by Harlan and DeWet (1971), Smartt (1979) proposed for introducing a quaternary gene pool to accommodate related species that form efficient genetic barriers but whose resources could be utilized by genetic engineering techniques in the future. *L. sativus* is the only member of the primary gene pool of *Lathyrus*. As a result, distant hybridization is presently used to harness and utilise germplasm resources for further improvement (Yunus and Jackson 1991). Secondary gene pool consists of 10 species *viz*., *L. cicera*, *L. amphicarpus*, *L. chrysanthus*, *L. gorgoni*, *L. marmoratus*, *L. pseudocicera*, *L. blepharicarpus*, *L. chloranthus*, *L. hierosolymitanus* and *L. hirsutus*. However, reports are meager about the cross compatibility of these species with the cultivated *L. sativus* to produce viable seeds which seeks urgency to exploit potential of utilizing these species in grass pea improvement program. Recent study by Heywood et al. (2007) stated that some of the members of the secondary gene pool are crossable and enable to produce fertile seeds with *L. sativus*. The remaining species belongs to tertiary gene pool which can be utilized for crop improvement program for transferring desired alleles in to cultivated background through deploying modern biotechnological tools like embryo rescue technique, etc. There are still ambiguities about the progenitor of *L. sativus* but Jackson and Yunus (1984) reported that *L. cicera* is morphologically and cytogenetically closely related to the cultivated species and it is most probably the progenitor of *L. sativus*.

9.4 Snapshots on Classical Genetics and Traditional Breeding

According to cytological and karyotype research in *Lathyrus* genus, most of the species are diploid and polyploidy is rare (Barpete et al. 2014; Hao et al. 2017). However, chromosomal size variation has been seen, which is primarily connected with 2C nuclear DNA amount fluctuation (Grando et al. 2010) or chromatin segment amplification or deletion during species diversification. In several cultivars of *L. sativus*, satellite chromosomes ranging from one to two pairs were found (Barpete et al. 2012). Beside chromosomal size variation, diversity has also been detected in the size and placement of secondary constrictions, as well as the centromere's location. Some aneuploid as well as polyploid species also reported among the *Lathyrus* genus *viz*., *L. palustris* ($2n = 6x = 42$, hexaploid) and *L. venosus*, ($2n = 4x = 28$, tetraploid) (Talukdar and Biswas 2008).

9.4.1 Mapping Efforts in Grass Pea

In grass pea, reports regarding morphological and cytological mapping is rare. During the initial phase of grass pea research program isozymes have been deployed for construction of genetic linkage map as the number of molecular markers were meager (Chowdhury and Slinkard 2000; Talukdar 2012). Only two isozyme loci, aspartate aminotransferase (Aat-2) and shikimate dehydrogenase (Skdh), were connected with a map distance of 28 cM among the 11 isozymes employed in Chowdhury and Slinkard's (2000) study for detection of their inheritance pattern and linkage. In legume like grass pea, Aat-2 can execute crucial role by acting as a precursor of essential amino acids methionine and lysine (Bryan 1980) as well as play key role in biosynthesis of ureides which are the major nitrogen transporter (Schubert 1986). Skdh, on the other hand, catalyzes the production of aromatic amino acids as well as a wide range of secondary metabolites in plants (Peek and Christendat 2015). Another study of linkage analysis with isozymes discovered that the loci inherited monogenetically with codominant expression (Talukdar 2012).

9.4.2 Breeding Objectives

With the growing popularity of grass peas, breeding programs have been refocused on improving plant architecture with appropriate phenology, resistance against biotic and abiotic stresses, product profiling for food and fodder purpose, high yield potential as well as good biomass using traditional breeding techniques (Hillocks and Maruthi 2012). Major efforts were only concentrated on development of low ODAP

cultivars (<0.1%) (Hanbury et al. 2000). As ODAP is related with abiotic stress tolerance in grass pea so it is pertinent to discuss about this in details. There are several reports regarding the inheritance pattern of ODAP content in seed of grass pea from Mendelian inheritance (Nerkar 1972) to presence of quantitative trait loci (QTLs) (Gowda and Kaul 1982; Tiwari and Campbell 1996). Reports are there about the presence of modifying genes (Quader 1985), presence of more than one gene (Briggs and Campbell 1990) as well as presence of maternal inheritance (Tiwari and Campbell 1996). A recent study discovered that the biosynthesis of ODAP involves more than two genes, with dominant alleles predominating (Tripathy et al. 2015). Genotype × environment interaction is reported regarding inheritance of ODAP content in grass pea (Nerkar 1972; Quader 1985; Tripathy et al. 2015). Therefore, negative selection is aided by soil nutrient status and environmental conditions such as drought, salinity, etc. which limit the expression of characteristics at the field level (Sarkar et al. 2019). In grass pea, reports are available regarding inheritance of flower and seed coat color, seed weight, nodulation and biotic stress resistances (Kumar et al. 2020). However, abiotic stress tolerance was less studied area in grass pea breeding program.

9.4.3 Breeding Achievements

Because of the neurotoxin stigma, very little work has been carried out for improving grass pea in recent past (Vaz Patto et al. 2006). Several breeding approaches like introduction, selection, hybridization, pre-breeding, distant hybridization and mutation breeding have been deployed in grass pea breeding programs (Dixit et al. 2016). Genotype with high yield potential as well as having good biological yield are the major focus (Abd El Moneim et al. 2001; Vaz Patto et al. 2006). Because grain yield is a quantitative trait, environmental influences, genotype × environment (GE) interactions, low heritability, nonadditive gene action, and negative trait linkages limit the efficiency of using grain yield as the exclusive selection criterion, making selection more difficult. Breeders rely on indirect selection for secondary traits that are easy to assess with highly heritable, and positively correlated with grain yield to overcome these challenges and achieve superior selection gains. As the systematic breeding efforts implemented by various National and International institutes, several high yielding varieties with low ODAP content have been developed (Table 9.2) (Abd El Moneim et al. 2001; Kumar et al. 2011; Dixit et al. 2016; Lambein et al. 2019). In India, Pusa-24, which was a field selection, considered as the first variety with low ODAP content in seed (0.2%) (Dahiya and Jeswani 1974; Lal et al. 1985; Mehra et al. 1995). Following this, research efforts resulted in the development of cultivars with low (up to 0.2%) ODAP concentration (LSD3, LSD6, Pusa-305, and Selection 1276) suitable to rice fallow niche of South East Asia (Gautam et al. 1998). Later on, varieties with high yield potential coupled with good harvest index *viz*., BioR-202, BioL-203, BioL-212 (Ratan), BioR-231, and BioL-208 have been developed (Gautam et al. 1998; Santha et al. 1998). The Indian land mark varieties Prateek (LS8246 × A-60) and Mahateora (BioL-212 × JRL-2) were developed through hybridization. These

Table 9.2 Grass pea varieties with low ODAP content under cultivation in different countries

Released/improved varieties	Pedigree/selection	Country	References
Pusa 24	Selection from germplasm	India	Lal et al. (1985); ICAR (2009); ICAR (2019)
Prateek	LS 82,046 × A 60		
Ratan	Somaclone of Pusa 24		
Mahateora	Ratan × JRL 2		
Nirmal	Selection from germplasm		
Bidhan Khesari-1	Selection from germplasm		
Bari Khesari 1	P-24 × Local	Bangladesh	Sarwar et al. (1996), www.bina.gov.bd
Bari Khesari 2	P-24 × Local		
Bina Khesari 1	Mutation		
Bari Khesari 3	Sel 190		
Bari Khesari 4	Sel 1337		
CLIMA 2 pink	Introduction	Nepal	www.acribd.com/doc/
19A	Selection		
20B	Selection		
Bari Khesari 2	Introduction		
Ceora	K33 × 8604	Australia	Siddique et al. (2001); Hanbury and Siddique (2000)
Chalus	Selection from IFLA 1279		
Luanco-INIA	Selection from LS 0027	Chile	Mera et al. (2003); Tay et al. pers. Comm 1983
Quila-blanco	Selection from germplasm		
Wasie (ILAT-LS-LS-B2)	SC5 x PGRC 46,071	Ethiopia	ICARDA (2007)
Ali Bar	Selection from IFLLS 554	Kazakhstan	ICARDA (2006)
LS 8246	Selection from Pusa 24	Canada	Campbell and Briggs (1987)
Strandja	Local selection (VIL)	Bulgaria	–
Derek	Selection from Der	Poland	Milczak et al. (2001)
Krab	Selection from Kra		
Gurbuz 1	Selection from IFLLS 554	Turkey	ICARDA (2007)
Studenica	Pedigree method of selection	Serbia	–
Stinica			

Source Adapted from Kumar et al. (2011, 2020)

have very low ODAP content (<0.1%) with yield potential of up to1.5 tons/ha and are still popular in North Eastern Plain Zone and Central Zone of India (Kumar et al. 2013; Dixit et al. 2016). ICARDA started extensive grass pea breeding program and in collaboration with national partners that resulted in 30 improved varieties which have been released and cultivated globally. In Ethiopia, one low ODAP grass pea variety 'Wasie' with yield potential of 1.7 tons/ha has been released. Similarly, 'AliBar' with yield potential of 1.2 tons/ha for dry areas has been released for cultivation in Kazakhstan (Kumar et al. 2020). In Turkey, 'Gurbuz 2001' has been released for its suitability to cultivate in the high altitude. Some of the varieties have been developed from the related species, *L. cicera* and *L. ochrus* like 'Chalus' and 'Ceora' in Australia, 'Jaboulah' in Lebanon, 'IFLLO 185' in Jordan and have been released for cultivation (Kumar et al. 2020). 'Chalus' has high protein (26.5%) and low ODAP (0.09%) content (Hanbury and Siddique 2000). Similarly, in Bangladesh, two varieties with good yield potential and low ODAP level (0.29%) namely, Barikhesari-1 and Barikhesari-2, were developed through hybridization (Malek et al. 1996). The cultivar 'Quila-blanco' was selected from a locally cultivated diverse population in Chile in 1983. Similarly, another variety 'Luanco-INIA' having white color seed coat was also developed through selection from local germplasm accession (Mera et al. 2003). Likewise, in Australia one variety i.e. 'Ceora' with low ODAP levels (0.04–0.09%) has been developed through hybridization between two parents from Pakistan and Bangladesh (Hanbury et al. 1995; Siddique et al. 2001). 'CLIMA pink', '19A', and '20B' in Nepal; 'LS 8246' and 'Derek' and 'Krab 9' in Poland, 'AC-Greenfix' in Canada; 'Gurbuz-1' in Turkey; and 'Strandja' in Bulgaria are some of the other popular cultivars with low ODAP concentration.

9.5 Brief on Diversity Analysis

Genetic diversity is the basis for response to selection and further achieving genetic gain over many years of selection. Presence of substantial amount of genetic diversity in a species will facilitate to acclimatize under new agroecological condition. Detailed information of its closest relatives and geographic origin are key milestones during selection of diverse parents in hybridization program (Schaefer et al. 2012). Genetic diversity can be assessed through morphological, biochemical and molecular markers. However, morphological parameters being qualitative or quantitative are impacted by both the environmental and genetic factors, they may not depict the true population/germplasm variability (Tanksley 1983). As grass pea is a under-researched crop so, most of the variation present in gene pool of grass pea germplasm are becoming extinct. Conservation and maintenance of genetic diversity is the prerequisite for sustainable crop improvement program. Several methodologies have been deployed to decipher the phylogenetic relationships among *Lathyrus* gene pool including morphological, biochemical and molecular markers (Vaz Patto and Rubiales 2014; Lambein et al. 2019).

9.5.1 Genetic Diversity Based on Morphological Traits

Plant species delimitation was once solely dependent on morphological traits which can be classified into monogenic or polygenic group. The expression of both oligogenic and polygenic traits is controlled by environmental factors, and these types of trait variations are commonly referred to as both genetic and environmental qualities. Therefore, individuals within the same species, on the other hand, may exhibit morphological variety, either spontaneously or as a result of local adaptations thus it will create some cryptic groups. Another drawback of morphological markers is that they cannot be accessible easily irretentive of growth, developmental stages or specific environmental condition.

Extensive genetic variation was reported in grass pea regarding leaf shape, flower color, duration of flowering, pod shape, seed size, seed yield and seed coat color which are mostly differed in various geographical location. Ample variation in agronomic traits of grass pea have been observed by several researchers (Deshpande and Campbell 1992; Polignano et al. 2005; Tarade et al. 2007). Plant height ranged from 15 to 172 cm, branches per plant varying from 1.8 to 40; pods/plant ranged from 2.4 to 59; days to flower initiation (34–62 days), days to 50% flowering (47–103 days), days to maturity (97–156 days) depending upon the country where the studies have been carried out (Campbell 1997; Pandey et al. 1995). Recently, ample number of variations for different morphological traits like plant height, days to flowering, days to maturity, seeds/pods, pods per plants, primary branches, grain yield and biological yield has been observed using descriptive statistics and cluster analysis (Parihar et al. 2013; 2015). In grass pea, flowers are of attractive color like blue, pink, red, white or various combinations. The blue-flowered ecotypes are found in South-East Asia and the South Asia with high outcrossing percentage (Polignano et al. 2005; Kumar et al. 2013); whereas, white flowered types with less outcrossing are generally prevalent in Mediterranean region (Smartt 1979). Considerable variation was also detected in pod size and seed coat color. Pods of grass pea are wrinkled, or round in shape and the seed coat color varies from ash, brown, grey, creamy or black. In grass pea, seed size is one of the key features of germplasm delimitation. Seed index of grass pea ranged from 2.95 to 22 g depending upon the environmental condition (Hanbury et al. 1995; Robertson and Abd El Moneim 1995). Generally, the large seeded type known as "lakh type" are common in Mediterranean region whereas the small seeded type known as "lakhori type" are prevalent in South Asia and Poland (Hanbury et al. 1995). Additionally, various nutritional or antinutritional parameters like protein, nutritive value and ODAP have been estimated among the *Lathyrus* species (Grela et al. 2010). Within the available germplasm, there was a wide range of ODAP content, ranging from 0.02 to 2.6% (Hanbury et al. 1999; Kumar et al. 2011). It was observed that grass pea genotypes from South East Asia and Ethiopia is having high ODAP (0.7–2.4%) in comparison to the germplasm sourced from Mediterranean region (0.02–1.2%) (Abd El-Moneim et al. 2000). In comparison to *L. sativus*, the ODAP concentration in *L. cicero* and *L. gorgoni* is lower among the wild species (Hanbury et al. 1999;

Kumar et al. 2013). This diversity is unquestionably the product of geographical separation as much as human selection.

9.5.2 Genetic Diversity Based on Biochemical and Molecular Markers

Biochemical markers like isozymes as well as DNA based markers are getting importance over the morphological markers for delineating closely related individuals (Duminil and Michele 2009). It was observed that induced dwarf mutants of grass pea have allozyme variants regarding root peroxidase and leaf esterase isozymes, as well as a relationship to dwarfing genes that can be effectively utilized for discriminating dwarf mutants from one another (Talukdar 2010). Chowdhury and Slinkard (2000) studied the inheritance pattern as well as linkage of 11 isozymes from eight enzyme family using four F_2 populations. Mendelian inheritance was observed for all the studied isozymes and two isozymes namely Aat-2 and Skdh were linked with 28 cM map distance. Three isozymes namely esterase, aspartate aminotransferase and acid phosphatase used for measuring variability among the Ethiopian grass pea resulted non-significant correlation with morphological diversity (Tadesse and Bekele 2003).

The number of functionally relevant molecular markers in grass pea is unexpectedly low, necessitating the development of a high number of functionally relevant molecular markers for successful deployment in molecular breeding strategies. In grass pea breeding program, diverse molecular markers like restriction fragment length polymorphism (RFLP); random amplified polymorphic DNA (RAPD); inter simple sequence repeat (ISSR); amplified fragment length polymorphism (AFLP); expressed sequence tag-simple sequence repeats (EST-SSR); sequence tagged site (STS) and sequence-related amplification polymorphism (SRAP) have been utilized for deciphering diversity and phylogenetic relationship among the species (Asmussen and Liston 1998; Hanada and Hirai 2003; Chtourou-Ghorbel et al. 2001; Nosrati et al. 2012; Lioi et al. 2011; Lioi and Galasso 2013; Ambade et al. 2015; Soren et al. 2015; Marghali et al. 2016).

9.6 Discovery and Application of Molecular Markers and Mapping Techniques in Grass Pea

Molecular marker is the stretch of DNA which is associated with the specific location of the genome. Molecular markers have a variety of applications like diversity study, genome mapping, gene identification as well as association mapping of quantitative traits (Grover and Sharma 2016; Liu et al. 2020). Markers as well as genes are available on chromosomes and they tend to stay generation after generation. Based on the availability of markers on the chromosome and the close proximity of a marker

with a gene, genetic linkage map can be developed and subsequently the association of different genes or QTLs with a particular trait can be monitored (Semagn et al. 2006). Majorly used molecular markers are RAPD, RFLP, ISSR, AFLP, SSR and single nucleotide polymorphism (SNP), etc. (Grover and Sharma 2016). Out of these molecular markers, SSRs and SNPs are widely used in plant breeding due to their reliability (Tsykun et al. 2017) but genomic information is needed for SSR as well as SNP analysis. Due to scarcity of genomic information in public database very lesser number of molecular marker-related studies have been conducted in grass pea in respect to abiotic stresses. Early work on molecular markers (AFLP and SSR) in grass pea was conducted using Italian grass pea cultivars (Lioi et al. 2011). During the same time period another diversity study on Ethiopian grass pea was conducted using EST-derived SSRs and cross transferable EST-SSRs from other legumes (Ponnaiah et al. 2011). In the following year Sun et al. (2012) developed novel EST-SSRs from 24 grass pea accessions collected from different parts across the world. The genomic database of grass pea is poorly developed compared to other pulse crops due to larger genome size (~8.2 Gb) of grass pea (Bennett and Leitch 2011) and it is the main reason for lesser availability of SSRs in grass pea. Subsequently, using genomic SSR, another informative molecular marker, several SSRs were developed from *L. sativus* those were found to be highly transferrable among related species like *L. cicera*, *L. ochrus*, *L. tingitanus*, as well as *Pisum sativum* (Lioi and Galasso 2013). Using the advanced molecular biology technique of 454 FLX Titanium pyrosequencing, enormous number of SSR loci were discovered and 288 SSRs were validated among different accessions of *L. sativus* and single accession of *L. cicera* (Yang et al. 2014). Further marker-related molecular studies were conducted in grass pea using transcriptomic data (Almeida et al. 2014a, b; Hao et al. 2017). In the detailed study conducted by Hao et al. (2017), 3,204 EST-SSR primers were identified and 43 grass pea accessions were validated using randomly chosen 284 EST-SSR primers. Additionally, 146,406 SNPs were screened and out of them arbitrarily chosen 50 SNPs were validated through kompetitive allele-specific PCR (KASP). This study led to the development of successful KASP markers from 42 SNP loci (Hao et al. 2017). Most recently, Soren et al. (2020) reported significant marker–trait associations using SSR markers for plant phenology and yield-related traits. The gene ontology revealed that the rubredoxin family protein, homeobox-leucine zipper protein ATHB-6-like and cationic peroxidise genes are associated with marker regions. This association of markers with novel traits expression would certainly play a significant role in crop improvement programs of grass pea. Although several researchers developed multiple SSRs and SNPs in grass pea, till now to our knowledge there is no significant impact of molecular markers in abiotic stress tolerance in grass pea.

Molecular markers have been used to create three linkage maps for any *Lathyrus* species so far (Table 9.3). A total of 71 RAPD markers were used to create the world's first linkage map with 14 linkage groups in grass pea utilizing 100 F_2 individuals resulting from a cross between a white flowered parent and a blue flowered parent (Chowdhury and Slinkard 1999). To detect linkage and generate linkage maps, MAPMAKER was used and map distances were calculated using the Haldane

Table 9.3 List of the QTL mapping strategies in Lathyrus/grass pea

Trait	QTL number	Mapping population	Marker system	References
Flower color marker	14 linkage groups	100 F2 individuals	71 RAPDs	Chowdhury and Slinkard (1999)
Stem resistance to ascochyta blight	09 linkage groups	92 backcrossed individuals	47 RAPD, 7 EST-SSR, 13 STS/Cleaved Amplified Polymorphic Sequence (CAPS) markers	Skiba et al. (2004)
Tendril	–	F_2 population	302 RAPD markers	Hanada and Hirai (2003)
Rust *Uromyces pisi*) resistance	–	RIL population	CAPS and derived-CAPS (dCAPS), monomorphic simple sequence repeats (SSR)	Almeida et al. (2014a, b)
Response to rust (*Uromyces pisi*) resistance	9 linkage groups	103 F5 Recombinant Inbred Lines (RIL) population derived by single seed descent	189 SNP, 113 EST-derived Simple Sequence Repeats (E-SSR), and 5 Intron Targeted Amplified Polymorphism (ITAP) markers	Santos et al. (2018)
Total phenolic content	–	100 accessions	Diversity Arrays Technology Sequencing (DArTseq) based SNP markers	Patto et al. (2018)

function (Lander et al. 1987). In another study, 92 backcross individuals resulting from a cross between Ascochyta blight induced by *Mycosphaerella pinodes* resistant accession (ATC 80,878) and susceptible accession (ATC 80,407) were used to create a linkage map with 9 linkage groups (Skiba et al. 2004). In the backcross population, a total of 64 markers were mapped, comprising of 47 RAPD, 7 sequence-tagged microsatellite sites, and 13 STS/CAPS markers for construction of a linkage map with a distance of 803.1 cM having nine linkage groups with an average spacing between the markers of 15.8 cM. Simple and composite interval mapping coupled with single point analysis were used for detection of QTLs associated with Ascochyta blight resistance. In temperature-controlled growing chamber, the backcross population was tested for Ascochyta blight resistance. Two QTLs (QTL1 and QTL2) were detected located on linkage group 1 and linkage group 2

respectively. The first QTL explained 12% whereas the second one clarified around 9% phenotypic variation in the backcross population. Another genetic linkage map of *L. cicera* in response to rust infection (*Uromyces pisi*) was developed by Santos et al. (2018) based on RNA sequencing-derived markers using recombinant inbred line (RIL) population developed from a cross between 'BGE023542' (resistant) and 'BGE008277' (susceptible). A total of 935 molecular markers including 767 SNPs, 163 E-SSRs, and 5 inter-retrotransposon amplified polymorphism (ITAP) markers were used in the study. The linkage map constructed in the study comprised of 307 markers with an average mapping interval of 2.4 cM, covering 724.2 cM, and is organized into 7 main and 2 minor linkage groups (Santos et al. 2018). Linkage analysis was performed using the JoinMap 4.0 software (Van Ooijen 2006). Recently, another genetic linkage map was constructed by the same research group for studying the presence of Mildew Locus O (MLO) gene family members conferring resistance against powdery mildew. The mapping population was 105 RILs in the F_5 generation from crossing between Raipur-4 × LS87-124–4-1. All together 163 E-SSR markers coupled with 767 SNPs, and five heterologous ITAP markers selected from previous publications were utilized for map construction. In this study also, JoinMap 4.0 software was used to perform linkage analysis and segregation distortion testing. A LOD score of 4 was used to identify groupings of connected markers.

Still, the discovery of large number of molecular markers and construction of highly statured genetic linkage map in grass pea is not in good shape which is the prerequisite for detecting the position of the genes and QTLs in the genome for paving the way in MAS program. Cloning and identification of novel genes conferring resilience to climate vagaries as well pest infestation followed by their introgression through MAS will facilitate development of improved cultivars in grass pea.

9.7 Genomics-Aided Breeding for Resistance Traits

Eukaryotic gene expression is controlled at various check points: at chromosome level, at transcript level, at post-transcriptional level, during translation as well as through epigenetic regulations. Being an under researched legume, genomic resources are poor in grass pea. Recently with the unprecedented breakthrough of NGS based technologies transcriptomic studies have also been initiated in grass pea (Yang et al. 2014; Almeida et al. 2015; Chapman 2015; Tan et al. 2017; Xu et al. 2018; Rathi et al. 2019). Transcriptome profiling has been carried out for unraveling grass pea-*U. pisi* interaction towards identifying the putative biochemical pathways and transcripts governing resistance in this food legume (Almeida et al. 2014a). Several hormonal pathways like salicylic acid, jasmonate and ethylene pathways and pathogenesis related (PR) proteins have been detected through this study which can shed light on legume resistance breeding. The same group also carried out transcriptome profiling of two cultivars of grass pea showing contrasting performance against Aschochyta blight incited by *A. lathyri* (Almeida et al. 2015). Here also, ethylene and salicylic acid pathways were detected as the key factors for

improving resistance. In grass pea the biosynthesis pathway of β-ODAP is still in ambiguity. Transcriptome study has been conducted to unveil the genes and pathways governing β-ODAP accumulation in different growth stages and concluded that the cysteine synthase genes influenced β-ODAP accumulation and were coregulated with primary metabolism (Xu et al. 2018). Only one transcriptome study has been carried out in grass pea in relation to abiotic stress tolerance and several transcript level variants have been detected concerning drought tolerance in this food legume (Rathi et al. 2019). Along with the genomic and transcriptomic progress, proteomic research has also been conducted on grass pea. A proteomic study conducted on grass pea identified about 100 protein spots through two-dimensional gel electrophoresis which were at least twofold differentially expressed upon exposure to independent treatment of salt stress, cold stress and abscisic acid treatment for 36 h compared to control plants (Chattopadhyay et al. 2011). Further identification of those proteins through LC MS/MS identified 48 stress responsive proteins and out of which 33 proteins were associated with all of those three stresses while the expression of 15 proteins were specific to individual stress.

9.8 Recent Concepts and Strategies Developed

During normal plant growth and development as well as upon exposure to various stresses, miRNA genes are expressed and subsequently miRNAs regulate the gene expression of a number of downstream genes either through mRNA degradation of the target genes or through translational regulation (Akdogan et al. 2016). A recent study on grass pea identified several miRNAs associated with drought tolerance. Among the identified known miRNAs, 8 miRNAs were upregulated under drought stress while 12 known miRNAs were down regulated under drought condition (Bhat et al. 2020). Other than the known miRNAs, a number of novel miRNAs were also identified in grass pea. Further studies are needed to functionally characterize different miRNAs available in grass pea involved in various stress signaling and development of transgenic plants to combat abiotic stresses through functional genomics approach.

Unlike the miRNA-mediated gene regulation, small interfering RNA (siRNA)-mediated gene silencing and virus induced gene silencing (VIGS) also regulates the gene expression only at post transcriptional level (Unver and Budak 2009; Kasai et al. 2011; Akdogan et al. 2016). Through siRNA or VIGS mediated gene silencing strategies, the double stranded RNA is generated within the targeted plant species using exogenous vector-based DNA molecule (Banerjee et al. 2017; Lee et al. 2017). An interesting study reported the VIGS-mediated silencing of phytoene desaturase (PDS) gene in *L. odorata* but till now to our knowledge no other *Lathyrus* sp. including grass pea documented successful VIGS-mediated gene silencing (Grønlund et al. 2008). Gene regulation through siRNA or miRNA often leads to off target effects and to get rid of that genome editing technologies are gaining significant attention among the scientific communities for specifically deregulate a target gene (Zhang et al. 2015). Generally, three approaches of genome editing are popular among

the researchers namely clustered regularly interspaced short palindromic repeats (CRISPR)-, transcription activator like effector nuclease (TALEN)- and zinc finger nuclease (ZFN)- mediated approaches having potential merits and demerits of each one of them (Zhang et al. 2019). Although, the draft genome of grass pea has been placed but unfortunately, till now no significant development has been done on grass pea genome editing for counteracting any abiotic or biotic stresses (Emmrich et al. 2020; Kumar et al. 2020). Another reverse genetic approach is TILLING introduced by McCallum et al. (2000) that combines both high density of point mutations generated by chemical mutagens like ethyl methane sulphonate (EMS) or through physical mutagenesis to produce deletions of various magnitude followed by rapid detection of the mutant to discover induced lesions (McCallum et al. 2000). Another approach for detecting natural variants is known as EcoTILLING (Comai et al. 2004). Grass pea is a good candidate for both TILLING and Eco-TILLING as the genomic resources are meager in this legume as well as it is recalcitrant in nature during genetic transformation. Initiative has been taken at John Innes Centre, Norwich, Norfolk, England for studying EMS mutagenized populations for searching low ODAP mutant (Emmrich 2017).

9.9 Transgenic Research on Grass Pea in Relation to Abiotic Stress

Grass pea is a lucrative leguminous crop possessing genes for several abiotic stress tolerance (Lambein et al. 2019). Unfortunately, in spite of having such exciting resistance gene pool the wider acceptance of grass pea is limited to human beings due to the presence of ODAP (Hoque et al. 1996; Kuo et al. 2000). Several studies were conducted on the ODAP estimation in grass pea collected from different locations but till now zero-ODAP or ODAP-free grass pea genotype has not been identified. Scientists have paid sufficient attention for understanding the molecular mechanisms associated with abiotic stress as well as ODAP biosynthesis in grass pea for subsequently manipulating the plants at genetic level to achieve target-oriented goal. For successful genetic manipulation of plants, suitable regeneration (Fig. 9.1) and transformation protocol should be optimized (Barpete et al. 2020). In vitro differentiation of shoot bud or regeneration through callus was optimized from various tissues like shoot tips, stem, leaf as well as root, seed and epicotyl explants and these were nicely reviewed by Zambre et al. (2002). Another attempt was made for grass pea transformation through biolistic gene gun method (Barna and Mehta 1995). Later on, prolific regeneration protocol of grass pea was established from green nodular callus developed from meristematic tissue (Zambre et al. 2002) and furthermore successful *Agrobacterium*-mediated transformation was conducted using epicotyl segment (Barik et al. 2005). In addition to that, another group reported *Agrobacterium rhizogenes*-mediated transformation in a different *Lathyrus* species (*L. maritimus*) and subsequently somatic embryogenesis was also documented from the transformed

Fig. 9.1 In vitro regeneration of *Lathyrus sativus* L., **a** nodal junction explant, **b** and **c** shoot initiation and multiplication form nodal junction explant, **d** root induction on nodal junction derived shoot of grass pea

tissue (Jiangbo and Jingfen 2002). Other than these previously mentioned successful transformation efforts in *Lathyrus* sp., no significant recent developments are available on grass pea transformation to our knowledge. Hence there is a big research gap available and scientific interventions are needed to address the optimization of transformation protocol in grass pea and successful generation of transgenic lines of reduced ODAP content as well as improved abiotic stress tolerance.

9.10 Brief Account on the Application of Bioinformatics as a Tool

Although the genome size of grass pea is large (~8.12 Gbp), due to the advancement of genomic and transcriptomic research since last couple of decades many genes have been identified from grass pea using bioinformatics as a tool. Biological data mostly in the form of nucleotides or polypeptides are utilized by several bioinformatics platforms to unravel a gene, identify the open reading frame (ORF) of a gene as well as to understand the coding DNA sequence (CDS) of that gene. Several researchers have started transcriptome profiling of different tissues of grass pea for identification of transcript sequences and subsequently prediction of gene ontology and pathway determination using bioinformatics approach (Chakraborty et al. 2017; Xu et al. 2018). Along with the gene expressional changes upon biotic stresses, transcriptomics study was also conducted for abiotic factors in grass pea (Almeida et al. 2014a, 2015; Rathi et al. 2019). In addition to that expressed seEST library, transcriptome study as well as pyrosequencing approaches identified various molecular markers in grass pea by deploying various bioinformatics tools (Ponnaiah et al. 2011; Almeida et al. 2014a, 2015; Yang et al. 2014; Hao et al. 2017).

9.11 Social, Political and Regulatory Issues

Despite of its immense potential, grass pea area has been declined in India and other countries of the globe. After an epidemic of neurolathyrism in Nepal and India in 1961, a restriction on the storage and sale of grass pea was implemented (Kumar et al. 2011). The national acreage has gradually decreased from 5.4 lakh ha to 3.8 lakh ha in India over the last decade as a result of this restriction. Similarly, grass pea was extensively grown in North West China's Gansu region before the 1960s, but a major drought in the 1970s produced a horrible famine and a serious neurolathyrism outbreak which caused ban in grass pea cultivation (Yang and Zhang 2005). The production of grass pea has increased in Bangladesh, and it is also grown in various states of India like Chhattisgarh, West Bengal, Madhya Pradesh, Bihar and Maharashtra (Fig. 9.2). In these regions grass pea are mainly consumed as green vegetables, whole pods are cooked directly and consumed as vegetable, split dahl and adulterated with chickpea besan. In Bangladesh and in the above said districts of India there was no further report of lathyrism despite of consumption of grass pea since long back (Singh and Rao 2013; Khandare et al. 2014). Neurolathyrism was investigated among the grass pea consumers and animals and it was revealed that addition of methionine or sufficient cereal supplementation having important role to protect the cell against oxidative stress and neurolathyrism symptoms (Getahun et al. 2003; Fikre et al. 2011). When grass pea is used as the primary constituent of the diet that accounts for at least 30% of the caloric intake for at least 3–4 months, this crippling but non-lethal condition becomes more prominent (Dixit et al. 2016).

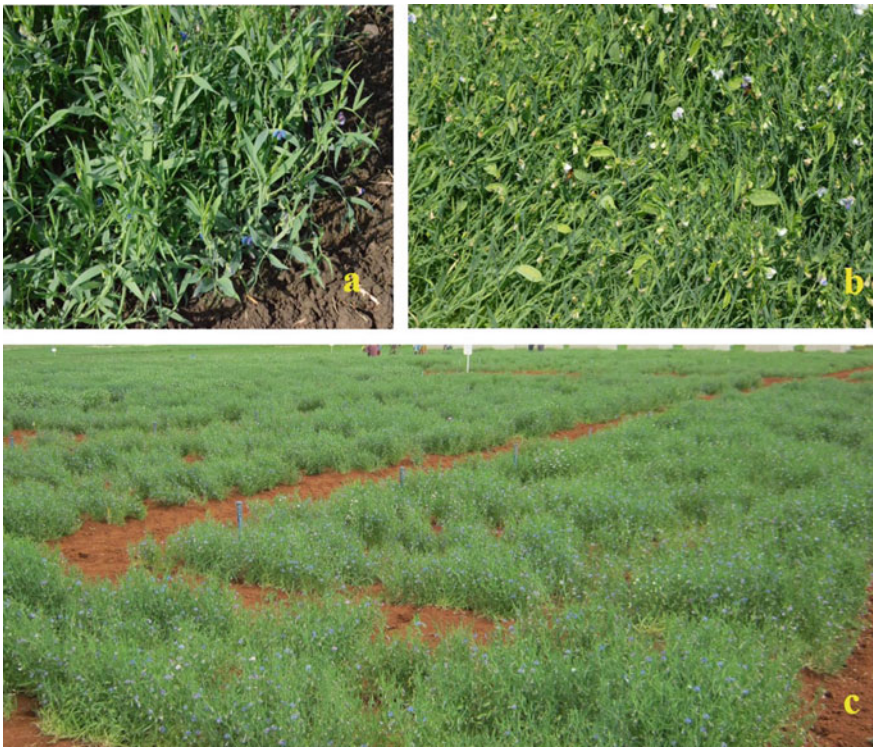

Fig. 9.2 Picture depicting Grass pea cultivation in India **a** vegetative and flowering stage, **b** podding stage, **c** grass pea crop in the field

However, several food processing strategies such as boiling in open pan for 90 mins (Barpete et al. 2021), soaking, roasting, or steeping in a 2% slaked lime solution for 3 h can partially detoxify seeds, and grains can also be toasted at 150 °C (Geda et al. 1995). In a meeting conducted on November 6, 2015, the Food Safety and Standards Authority of India (FSSAI) suggested lifting the prohibition on the storage and sale of grass pea and promoting the cultivation of low ODAP cultivars (Utkarsh 2016). However, being an often-cross pollinated crop with 27 to 36% outcrossing rate the gene flow from high ODAP varieties to low ODAP varieties cannot be undermined. There is pressing needs towards recommendation of an acceptable daily limit of grass pea for its safe consumption. It is high time to change the entire perception of this pulse and neurolathyrism and declare this pulse as "golden pulse of future".

9.12 Future Perspectives

With the increasing concerns about the potential consequences of climate change on agriculture have resulted paradigm shift towards focusing on under-utilized crops instead of the major crops in the last decade. Grass pea holds great promise as a food and feed in the resource poor marginal lands. Grass pea with low carbon, nitrogen and water foot print, is already a climate resilient crop with unique traits and trails of good agronomic features that enable resistance against problem soils, temperature extremities, water limiting situation and other climatic vagaries. In many instances, grass pea is the only nutrient sources in severe drought and famine thus it can be stated as a "mankind savour crop". With good nutritional profiling and presence of unique homoarginine grass pea is considered as a functional food. Despite of immense potential, grass pea is the most under researched legume due to the stigma associated with the presence of ß-ODAP which lead to declining area and cultivation under this crop. Grass pea has not received systematic breeding efforts and world-wide very few scientific communities are associated with grass pea improvement program and investment is also insignificant in comparison to other legumes like chickpea, pigeon pea and lentil. The major focus in grass pea breeding program should be on development of low ODAP containing cultivars with good agronomic base. Though, with concentrated breeding efforts several high yielding varieties with low ODAP content has been developed in the recent decade which resulted substantial increase in the grass pea cultivation in South Asia, Mediterranean region, Ethiopia etc. As an alternative to low ODAP cultivars, remodeling quality features that can mitigate ODAP's detrimental impacts should be considered. These include boosting homoarginine, cysteine, or methionine content to mitigate the problem of neuro-lathyrism. Utilization of potential CWRs and tapping of valuable genes from the gene pools are not received considerable efforts in grass pea breeding programme which seeks urgency to exploit these valuable sources of novel alleles conferring resistance against biotic and abiotic stresses followed by their introgression in cultivated background. Genomic resources in grass pea are also very meager. Recently with the unprecedented progress in NGS technologies, transcriptome profiling generated a good number of molecular markers and putative candidate genes associated with ODAP biosynthesis pathway, drought tolerance, Aschochyta blight resistance etc. in grass pea that will facilitate precision breeding like MAS, mining and cloning of candidate resistance genes of concern. High throughput phenotyping and genotyping facility in grass pea will promote gene discovery through genome wide association mapping. Genome sequencing of grass pea is under progress with already published draft genome sequences (Sarkar et al. 2019). The data will allow gene annotation and discovery of novel biosynthetic pathways like ODAP, protein etc. which is still in ambiguous stage in grass pea. Integrated OMICS approach will enable confirmation of the sequencing results at functional level. Cutting edge tools like TILLING and different genome editing approaches are critically needed to strengthen grass pea breeding program. Collaborative research efforts in National and International level are imperative for turning the crop from 'orphan' to' main stream' legume.

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